UNIVERSITY OF ALBERTA

TECHNOLOGY, COMPOSITION AND PROPERTIES OF DRY MILK PROTEIN CONCENTRATES PRODUCED BY ULTRAFILTRATION

by

JACQUELINE GELFAND ©

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IN

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DEPARTMENT OF AGRICULTURAL, FOOD
AND NUTRITIONAL SCIENCE

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Jacqueline Gelfand

8103-70 Avenue
Edmonton, Alberta
T6C OA6

Date: 30 September, 1996

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled TECHNOLOGY, COMPOSITION AND PROPERTIES OF DRY MILK PROTEIN CONCENTRATES PRODUCED BY ULTRAFILTRATION submitted by Jacqueline Gelfand in partial fulfillment of the requirements for the degree of Master of Science in Food Science and Technology.

.....
Dr. Lech Ozimek (Supervisor)

.....
Dr. Willem Sauer

.....
Dr. Dave Schroder

.....
Dr. Mark Michalak

Date: September 27, 1996

ABSTRACT

Research was conducted to manufacture milk protein concentrate (MPC) powders with different levels of protein and lactose by ultrafiltration and diafiltration. Then the specific aims were: 1) to study chemical composition and properties of MPC; 2) to establish the heat classification of MPC based on the determination of whey protein denaturation; 3) to evaluate water sorption isotherms and surface hydrophobicity of MPC; and 4) to determine the heat stability of MPC manufactured by ultrafiltration.

Protein concentration in MPC increased from approximately 33 to 87 % (dry matter) whereas lactose concentration decreased from 58.11 to 2.55 % when milk was ultrafiltered and diafiltered. The heat treatments of skim milk at 72 C/16 sec (low heated) and 90 C/5 min (high heated) resulted in MPC that contained on average 135 and 4.13 mg undenatured whey proteins (UWP) per g of total proteins, respectively.

Water sorption isotherms for MPC were sigmoidal resembling type II BET classification. Significantly ($P<0.001$) higher surface hydrophobicity was observed in the high heated MPC than in low MPC. The heat coagulation time (HCT) of skim milk decreased during concentration of milk proteins by ultrafiltration and was characterized by type A behavior.

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I never thought today I will be working in Canada or studying dairy when I first started my carear in food science. I have enjoyed very much what I learned through this research and it has left me with a great desired to continue exploring this area. However, without the support of friends and family this would have not been possible.

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Finally, I like to express my gratitude to my husband Jan Gelfand for his love, patience and support every step of the way. Jan has been my best companion and friend who always has believed and encouraged me to finished. I would also like to thank my parents and sisters for their love and effort to bring me to where I am today.

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CHAPTER 1

1. Introduction

This chapter provides background information relevant to the projects undertaken in presented studies.

Milk is a source of energy, essential fatty acids, amino acids, minerals and vitamins and is regarded as an essential nutrient of high biological value. Consequently, milk has an obvious nutritional role in the human diet. It is important to preserve the nutritive value of milk during the manufacture of dairy products. Processing techniques such as heat treatment, homogenization and acidification affect basic properties of the milk components and hence the character of the final product. New technical advances such as membrane processing are opening doors to exciting new product development without altering the nutritional value of milk and at the same time taking advantage of the exceptional functional technological properties of the proteins.

Traditionally, milk is consumed in its fluid form, as well as through its conventional products such as cheese, fermented products and evaporated milk. The dairy industry's objective still remains to produce large supplies of milk and milk-based foods. However, consumer perceptions of what constitutes healthy food is changing. Therefore, the development of new sophisticated products with high value added and nutritional content is essential for further growth of the dairy industry as the market for traditional dairy products reaches peak levels. Milk protein concentrates and isolates with high incremental value have a wide field of application in the dairy industry as well as in other sectors of the food industry.

Milk proteins can be used to improve the quality of dairy products, bakery products, sweets and candies, soups and sauces, beverages, meats and processed cheese. For example, skim and whole milk, and whey are converted into milk powders and are used as food ingredients or as animal feed. In addition, different grades and types of casein, caseinates, whey protein concentrates and isolates are available today with different levels of protein, lipids and mineral compositions which are also used as food ingredients. However, the majority of these products are not differentiated for specific applications (Harper, 1992). Skim milk concentrates present the opportunity for using larger proportion of milk dry matter for food products with specific targeted application.

The use of ultrafiltration in the dairy industry is a useful tool for the diversification of the manufacture of milk products. Ultrafiltration is a process governed by molecular size, and hence in milk, reduces the concentration of low molecular weight substances increasing the relative concentration of protein and fat (Glover, 1986a). Membrane processing such as ultrafiltration provides the dairy industry with innovative ways of utilizing milk proteins by fractionating and concentrating valuable components (Glover, 1982; Jimenez-Flores and Kosikowski, 1986b). The manufacture of total milk protein concentrates with reduced lactose content by means of ultrafiltration and diafiltration presents an alternative to tailored products for specific applications in the food industry.

1.1. Chemical Composition of Milk

Milk is composed of water, fat, protein, carbohydrates, minerals and enzymes. Table 1.1.. illustrates the concentration and physical state of the milk components. The whey proteins, lactose and part of the minerals are present in milk in true solution whereas

casein is present in the form of colloidal suspension and milk fat in the form of emulsion. The composition and physical state of milk components make it very suitable for membrane fractionation and concentration. The diameter of basic milk components are shown in Table 1.2. and their differential sizes in the design of membrane separation technology.

1.1.1. Milk Proteins

Proteins are strings of amino acids joined by peptide bonds. It is known that there are four recognized levels of protein structure. The primary structure is the sequence of amino acids in peptides/proteins; the secondary structure is the coiling and arrangements of peptides; the tertiary structure is concerned with supercoiling; and the quaternary structure is the organization of more than one polypeptide chain into a polymer or aggregate structure. The sequence of amino acids in a protein determines its structure, conformation, and properties. Polypeptides containing more than 100 amino acids may assume various spacial arrangements in space. Each species of protein in the native state exhibits a unique three-dimensional structure that determines its biological and functional properties. Covalent and non covalent interactions are the structural forces responsible for maintaining three-dimensional structures.

Van der Waals interactions, hydrogen bonding, electrostatic interactions and hydrophobic interactions are the forces that are involved in the formation of structures of proteins and are related to their functional properties. (Kinsella, 1984). Hydrophilic amino acids of the peptide chain are oriented towards the outside of the molecule to interact with water whereas the hydrophobic amino acids are buried in the interior of the molecule.

Table 1.1. Chemical composition and physical state of milk components.

Constituent	Composition
Water (liquid continuous phase), %	87
Lipids (emulsion phase)	
Milk fat (triglycerides), %	3.80
Phospholipids, %	0.02
Carotenoids, mg/100g	0.04
Vitamins A, D, E and K, mg/100 g	0.02
Proteins	
Casein (colloidal dispersion), %	
αs1 - casein	1.00
αs2 - casein	0.26
β-casein	0.93
κ-casein	0.33
χ -casein	0.08
Whey proteins (solution), %	
β-lactoglobulin	0.32
α-lactalbumin	0.12
Blood serum albumin	0.04
Immunoglobulin	0.08
Proteose - peptone & others	0.06
Enzymes:	
Lactoperoxidase and triacylglycerol lipase in milk serum; acid phosphatase, alkaline phosphatase and xanthine oxidase are associated with milk fat globule membrane and proteases associated with casein micelles	

Constituent	Composition
Lactose (solution), %	4.60
Minerals and organic salts (true solution), mg/100g	
Ca	37.00
Mg	7.50
P	134.00
K	134.00
Na	46.00
Cl	106.00
Citrate	160.00
Bicarbonate	10.00
Minerals and organic salt (colloidal form), mg/100g	
Ca	80.00
P	95.00
Citrate	14.00
Mg, K, Na,	15.00
Non protein nitrogen (true solution), mg/100g	
Peptides	20.00
Amino acids	30.00
Urea	30.00
Water soluble vitamins, mg/100g	
B vitamins	20.00
Ascorbic Acid	2.00

Adapted from Walstra and Jenness (1984) and Jenness and Patton (1959).

Table 1.2. Dispersion of milk components.

Dispersion	Molecular Weight	Particle Size	Milk System Components
solvent			water
	100 D	0 nm	
	ionic	reverse osmosis	
true solution			lactose, salts in solution
	1,000 D	1 nm	
	molecular	ultrafiltration	
colloidal solution			whey proteins albumins, globulins soluble casein colloidal phosphate
	10,000 D	10 nm	
colloidal suspension			casein micelles (30-300 nm) calcium citrate
	100,000 D	200 nm	
	macromolecular		
suspension			bacteria (cocci)
		1 µm	
emulsion			bacteria (rods)
	500,000 D		fat globules
	cellular + microparticulate	10 µm	
			foreign particles
		30 µm	

Adapted from Riel (1985)

In assuming this configuration the protein expends the least amount of free energy to maintain its configuration. (Kilara et al., 1986). Milk proteins are present in colloidal suspension (casein) and solution (whey protein). They have been identified and classified as α_{s1}-casein, α_{s2}-casein, β-casein, κ-casein, α-lactalbumin, β-lactoglobulin, serum albumins, immunoglobulins (G_1, and G_2), and secretory components.

In bovine milk there are two major type of proteins, each defined by their behaviour in acid environment. The caseins are defined as those phosphoproteins that precipitate at pH 4.6 (isoelectric point) at 20° C (Eigel et al, 1984). The proteins remaining in the solution are the whey proteins. The whey proteins can be subdivided into major mammary-synthesized proteins such as α-lactalbumin and β- lactoglobulin and minor blood proteins such as immunoglobulins, serumalbumins and proteose-peptone fraction.

1.1.1.1. Caseins

Caseins account for 80% of total milk proteins. In milk, the caseins, together with calcium and phosphate occur mainly in the form of a large colloidal peptides called casein micelles. Each casein micelle consist of submicellar particles containing hydrophobically associated α_{s1}-casein, α_{s2}-casein, β-casein, κ-casein in a ratio of 40:10:35:12 (Fox, 1989). At the natural pH of milk (6.8-6.9) the micelles are colloidally, electrostatically, and sterically stabilized by calcium cross-bridging and coating by κ-casein (Kinsella, 1984a).

Caseins are mostly random coil polypeptides, with a high degree of molecular flexibility (Moor, 1982). Caseins are rich in phosphoseryl residues and therefore, individual casein molecules and casein aggregates show avid calcium binding properties,

are sensitive to pH, and have multiple hydrophilic and hydrophobic regions (amphiphilic properties), regions of high negative charge, and yet other regions that can serve as a substrate for enzyme action. Caseins precipitate out of milk at pH 4.6 (isoelectric point) and this physico-chemical characteristic has been used in the production of different casein products and in cheese manufacturing.

The α_{s1} and α_{s2}-caseins have five (A,B,C,D and E) and four genetic variants respectively. α_{s1}-casein contains eight phosphoseryl residues whereas α_{s2}-caseins contains 10 to 13 phosphoseryl residues and is the least hydrophobic of all the caseins. α_{s2}-casein sulfur containing aminoacids that may interact with β-lactoglobulin upon heat treatment of milk (Fox, 1989).

β-casein is a major milk component constituting approximately 28% of total milk proteins (Idolo and Ng-Kwai-Hang, 1992). β-casein consist of seven genetic variants and has five phosphate groups and is the most hydrophobic of the caseins. The C terminal region is highly hydrophobic and imparts pronounced surface active properties to β-casein. In food systems the amphiphilic nature of β-caseins plays an important role as a food emulsifier and stabilizer (Fox et al, 1983).

β-casein precipitates only in the presence of calcium ions at temperatures below 18°C. It is the most hydrophobic of all milk proteins and its characteristics are the most temperature dependent. β-casein dissociates from the casein micelle at 4 °C storage temperature (Fennema, 1985).

Two genetic variants have been identified for κ-casein. κ-casein has only one phosphoseryl residue and does not bind calcium strongly, it also has a relatively high solubility in dilute calcium solutions compared with α_s and β-casein (Kinsella, 1984). κ-

casein protects the micelles against excessive association and precipitation. κ-casein is the only glycosylated member of the casein group. Being a glycoprotein with a large apolar domain, κ-casein is quite surface active. The C terminal is very polar, charged and hydrophilic.

Caseins associate via hydrophobic bonding which plays a significant role in functional properties such as viscosity, gelation, water sorption, foaming and emulsification (Fox, 1989). Casein micelles are stable to physical forces and heat. In dried milk powders the micelles retain much of their native function and are therefore able to absorb significant amounts of water and when heated to high temperatures, set into a viscous gel (Kinsella, 1984).

The ionic strength of caseins affect their behavior in food systems. For example, by varying calcium levels the hydration capacity and surface activity of milk powders and caseins can be altered.

1.1.1.2. Whey Proteins

Whey proteins which account for 20% of total milk proteins are composed of β-lactoglobulin (~66 %), α-lactalbumin (~22%), immunoglobulins (~10%), bovine serum albumin(~6%), lactoferrin and enzymes. Whey proteins are globular and different from caseins in structure and properties (Kinsella, 1985). The physical state of whey proteins has a significant influence on the application of these proteins in foods.

β-lactoglobulin is the major milk protein representing 50% of the whey proteins. It consists of 162 amino acid residues with a calculated molecular weight of 18,277. The most abundant whey protein exists as a dimer consisting of a two identical subunits. Two

genetic variants have been found, A and B (Fox, 1989). The thiol group is important since it appears to facilitate R-SH/SS interchange reactions allowing the formation of new structures or intermolecular disulphide-bonded dimers and polymers upon heating and the rate of reaction increases above pH 6.8 (Mckenzie, 1972).

The conformation of β-lactoglobulin is pH and temperature sensitive and these characteristics are of technological importance. Undenatured, native β-lactoglobulin is noted for having excellent emulsification capacity as well as whipping/foaming properties. In its denatured form, β-lactoglobulin can form gels.

1.1.2. Minerals

The minerals in milk which account for about 1%, are chlorides, phosphates and citrates of potassium, sodium, calcium and magnesium. These minerals are mainly in the form of soluble salts (White and Davies, 1958). The potassium, sodium and chloride salts are in solution, whereas phosphates, calcium, magnesium and citric acid are partly in solution and partly in suspended colloidal form when bound to caseins (Weeb *et al*, 1983).

Colloidal calcium phosphate is essential in stabilizing the casein micelle structure in milk and plays a very important function in processing of dairy products and final product quality (Kiesker, 1977). For example, calcium plays a role in the heat stability of milk. The heat stability of concentrated milk can be affected by the mineral concentration, especially Ca content (Kocak, 1985).

The solubility of tricalcium phosphate decreases with an increase in temperature. Srilaorkul *et al* (1989) reported that there was an increase in buffer capacity of concentrated milk due to the increase of milk salts and proteins in the retentate and the

maximum was at pH 5.4. Removal of colloidal phosphate from casein occurs at pH 5.4.(Walstra and Jennesss, 1984; Kirchmeier, 1980).

1.2. Ultrafiltration and Diafiltration

1.2.1. Definition

Ultrafiltration is a pressure driven membrane process used to separate and concentrate components in solution based on their molecular size, charge, shape and affinity for the membrane. Ultrafiltration membranes are porous in nature with a primary role to act as a selective barrier. During ultrafiltration molecules of bigger sizes than the membranes are retained while those molecules smaller than the smallest pore are completely permeable (Cheryan, 1986). The osmotic pressures required during the process of UF are very low (500 kpa) because, the size of the molecules separated are relatively large (Jameson, 1984).

Milk is separated into two fractions: retentate and permeate (Figure 1.1). The retentate (concentrate) contains mostly fat and proteins. The permeate contains water and water soluble substances such as lactose, dissolved salts and non protein nitrogen. Those minerals bound to proteins are retained and concentrated during ultrafiltration while the minerals in solution pass through the membrane (Geen et al., 1984).

Diafiltration is the process by which low molecular weight components are removed by the solvent addition to the retentate and removal of water through an ultrafiltration membrane (Figure 1.2). Ultrafiltration can be followed by diafiltration against deionized water to remove residual lactose and minerals from the retentate fraction and increase protein concentration. (Harris et al, 1989 and Tratnik, 1991). The efficiency

of producing concentrated milk products can be increased by using membrane processing technology (Eckner et al, 1992).

1.3. Effect of Heat Treatment on Milk Proteins

Temperature treatment of milk and milk products is an integral part of technological process. Heat causes damage in milk proteins, minerals and affects the functionality of milk proteins. During thermal processing different milk proteins are denatured at different temperatures (Estelle et al., 1988). The order of denaturation of proteins in milk is immunoglobulins > serum albumin > β-lactoglobulin > α-lactalbumin. Environmental factors such as minerals concentration, pH and temperature affect the degree of protein denaturation. Casein micelles when compared to most of the other food proteins are very heat stable at pH 6.7, however, the stability decreases when the pH is decreased towards isoelectric point. In addition, the soluble calcium level in milk is pH dependent and affects the stability of the casein micelle (Brule et al, 1978). Whey proteins on the other hand are rapidly denatured by heating above 65°C followed by aggregation and denaturation (Southward et al, 1988). When β-lactoglobulin (β-lg) is denatured free thiol groups are exposed and new disulfide bonds may be formed within and between β-lg molecules and with κ-and α_s casein. (Mckenzie, 1971; and Smits and van Brouwershaven, 1980).

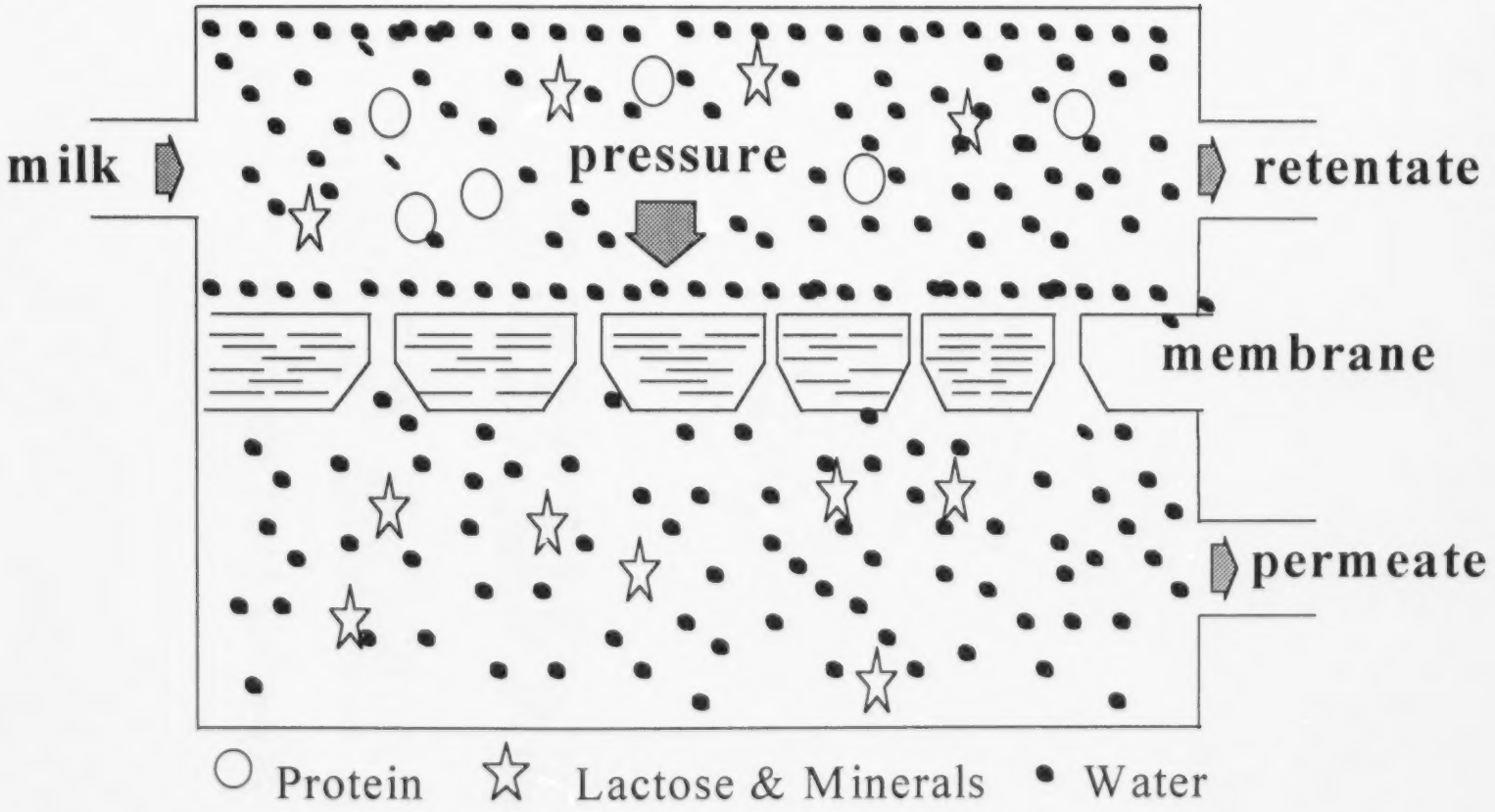

Figure 1.1. **Diagram of the ultrafiltration membrane process.**

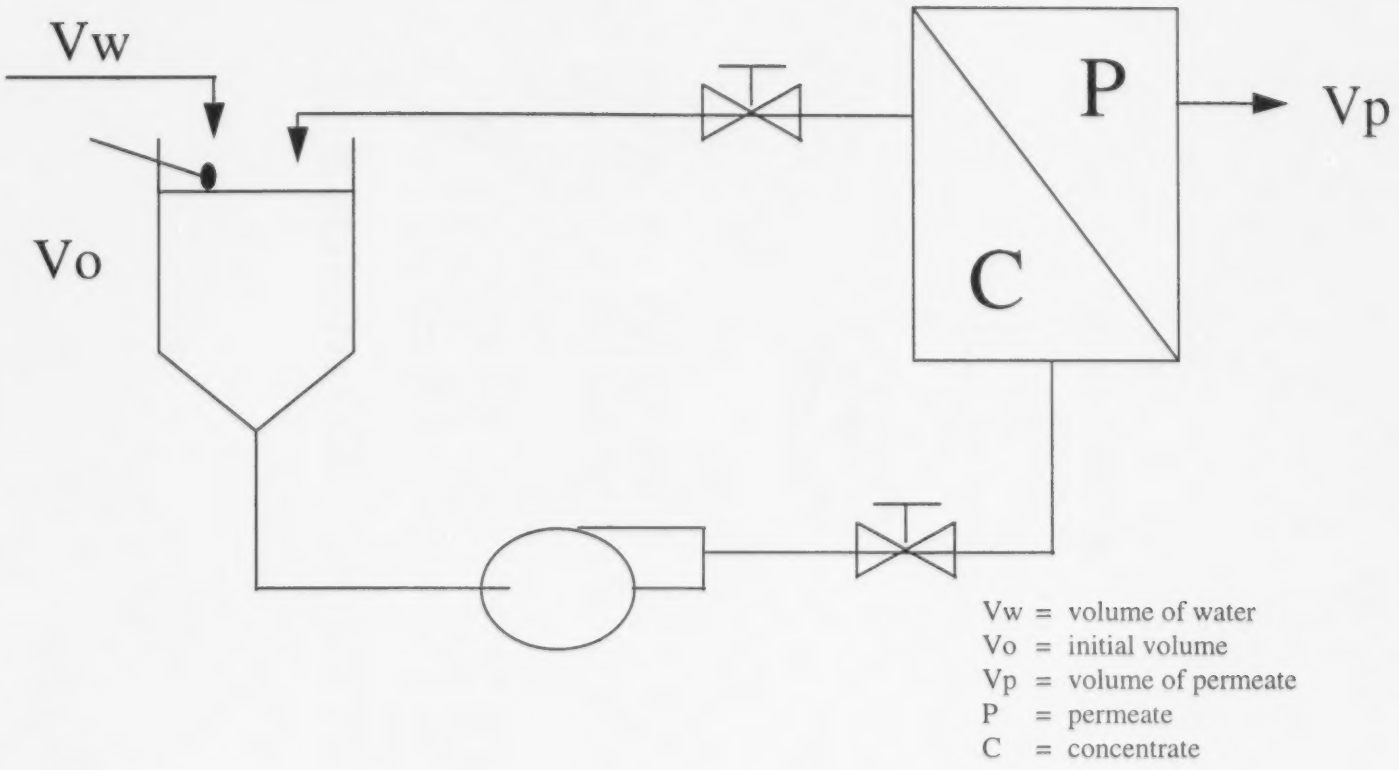

Figure 1.2. Schematic diagram of the diafiltration process with continuous addition of water.

1.4. Application of Milk Proteins in the Food Industry

Non fat dry milk is used widely in the food industry as an ingredient because of its great functional properties. However, the development of new manufacturing technologies has made it possible to produce milk powders with adjusted composition designed for specific applications. It is fundamental that both suppliers and manufacturers know the composition of milk powders according to official standards in order to apply it to their specific needs.

Depending on the environmental and processing factors, milk proteins can impart a wide range of physical properties to the foods in which they are contained. Milk powders are classified in extra and standard grades based on flavor, odor, physical appearance, bacterial load scorched particle content, lipid content, dispersability, solubility index, acidity and moisture content (Pallansch, 1970). In addition, heat treatment classification of milk powders is set accordingly based on the degree of denaturation of serum proteins. High, medium and low heated milk powders should contain <1.5, 1.5 to 5.9 and >6 mg of undenatured whey protein nitrogen (UWPN) per gram of non fat dry matter, respectively (Anon, 1971).

In addition to knowing the composition and heat classification of milk powders, it is important to understand their functionality in mixed and modified food systems where a combination of dairy and non dairy ingredients are used. For example, in the baking industry, the application of dry skim milk produced from milk heated to 80°C for 30 min was superior to low heated milk. The interaction of κ-casein and β-lactoglobulin is directly correlated to the increase in loaf volume (Guy, 1970). Other studies have attributed the

weakening of mechanical strength of the dough hence reducing its gas holding capacity to the presence of thiol groups (Kinsella, 1984). Low heat non fat dry milk powers are used in breakfast beverages because of their dispersion and hydration abilities (Webb, 1970).

Therefore, in order to commercialize milk protein products, the knowledge of their composition and structural properties and functional attributes is necessary to offer the food industry ingredients that are clearly differentiated from competitors and focuses on specific applications.

1.5. Functional Properties of Milk Proteins

1.5.1. Definitions

Functional properties can be regarded as the manifestation of the physical, chemical and conformational properties of the protein to influence the structure, appearance, texture, viscosity, mouthfeel, or flavor retention of a product.

Functional properties of different proteins are influenced by composition, structure, environmental conditions, prior treatments, and processing conditions. For example, functional properties are influenced by pH and ionic strenght as well as concentrations of sugars, stabilizers, emulsifiers, and lipids in the food product. In addition, the type and order of procedures employed such as freezing, concentration, dehydration, whipping and emulsification influence the functional properties of proteins.

According to Morr (1993) the majority of the functional properties of proteins may be classified into two main groups: hydrating-related and surface-related properties. Hydration-related functional properties include dispersability, solubility, swelling, viscosity, and gelation. Surface-related properties include emulsification, foaming and

absorption at air-water and oil-water interfaces. Other functional properties that do not fit into these two categories include diffusion, molecular unfolding (denaturation) and protein-protein, protein-ion and protein-ligand binding.

1.5.2. Hydrophobicity

Milk proteins individually offer a wide diversity of physico-chemical properties hence potential functional properties. The stability and conformation of milk proteins influence the emulsifying activity, texture, viscosity, and foaming capacity of the products in which they are found (Kinsella *et al.*, 1994). Similarly, the reactivity of the proteins in relation to the other macromolecules in solution contributes to the qualitative characteristics of food items. Hydrophobic interactions are largely responsible for these observations (Kinsella *et al.*, 1994; Ozimek *et al.*, 1994; Kato and Nakai, 1980).

Total hydrophobicity is described as being the sum of the non-polar amino acids in the protein. However, this analysis will not accurately account for the functional properties of the protein. Most of the hydrophobic sites are found "inside" the complex tertiary structure of the protein defining it's shape, but not having any interaction with other proteins or macromolecules in solution (Ozimek *et al.*, 1994). The analysis of a protein's surface hydrophobicity (S_o) is more useful, as it gives a direct indication of the interactions that the protein has with other molecules in solution (Keshavarz and Nakai, 1979).

Heat treatment of dairy products has a profound effect on hydrophobicity of milk proteins, and consequently on the functional properties that they display. The S_o is ultimately affected by the temperature of the milk, which determines the degree of

denaturation of the protein (Bonomi and Iametti, 1988). Theoretically, more hydrophobic sites will be exposed when protein is denatured. However, that may not be apparent if different proteins aggregate together to shield these newly exposed nonpolar sites (Bonomi and Iametti, 1988).

1.5.3. Water Sorption

The amount and nature of water that is found in food products has an important influence on the functional characteristics of the food components - principally proteins. Proteins and water interact on a variety of levels; for example absorptive, adsorptive, and hydrophobic levels. The water adsorption of proteins is instrumental in the functionality of the milk proteins in food products (Ozimek *et al.*, 1992; Kinsella, 1984). Adsorption specifies the adherence or binding of the surface of a solid to it's environment (Kinsella and Fox, 1986). In this case, adsorption refers to the adherence of water to the *surface* of the milk proteins. Water is strongly bound to the hydrophilic charged and polar amino acids of a protein. It has an enthalpy of vaporization considerably higher than pure water, is mostly unfreezable, and may not be available for chemical reactions (Kinsella and Fox, 1986).

Chemical deterioration, browning, gradual loss of solubility, lactose crystallization and the rate of rehydration are all affected by moisture adsorption of milk protein (Kinsella, 1984). For the average consumer, these characteristics are seen realistically as a reduced shelf life for dairy products, flavor alterations and a tainted overall mouth feel (Kinsella and Fox, 1986).

Thermodynamically, the degree to which the protein's surface layer is saturated with water can be measured as a function of water activity (Aw). This is done by applying the water sorption-desorption isotherm technique. Although the shape of the isotherm is dictated by each individual food product, sigmoidal isotherms are habitually obtained (Kinsella and Fox, 1986). Low A_w (less than 0.3) indicate that water is selectively attracted by hydrogen forces to charged and polar residues to form a monolayer of bound water. This water is unavailable for use as a solvent, but may be available for chemical reactions (Kinsella, 1984). As the water activities increase (0.3-0.9), multilayers of water build up beyond this monolayer, and eventually bulk water begins to associate loosely with the protein around its various polypeptides. This represents the major source of water in foods (Kinsella, 1984).

The development of new dairy products with modified protein and lactose levels by ultrafiltration/diafiltration requires characterization of water sorption isotherms.

1.6. Research Objectives

Traditionally skim milk powder is produced by evaporation of pasteurized milk and spray drying. The final product, dry skim milk powder contains all the dry matter component in the same proportion as in initial fluid milk. However, the introduction of membrane processing technology (ultrafiltration, diafiltration, nanofiltration, reverse osmosis) to milk processing creates great opportunities for the development of new dairy products with modified compositions. The overall purpose of the study was to apply membrane technology to manufacture milk protein concentrate powders with different

levels of proteins and lactose and to characterize their composition, properties and overall quality.

The objectives of the studies were:

1. To manufacture milk protein concentrate powders with different protein content between 50 to 85% from skim milk by ultrafiltration and diafiltration.
   a. To study the chemical composition of low and high heated dry milk protein concentrates manufactured by ultrafiltration and diafiltration.
   b. To determine whey protein denaturation and establish the heat classification of milk protein concentrates powders manufactured from skim milk by ultrafiltration and diafiltration.
2. To evaluate surface hydrophobicity and water sorption isotherms of low and high heated milk protein concentrates manufactured from skim milk by ultrafiltration and diafiltration.
3. To determine the heat stabilitly of milk protein concentrate powders manufactured by ultrafiltration.

This thesis is presented in the paper format following guidelines of the Faculty of Graduate Studies and Research, University of Alberta.

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Chapter 2

2. Technology and Properties of Milk Protein Concentrate Powders Produced by Ultrafiltration.

2.1. Introduction

The food ingredient market is highly competitive and continuously growing. Within this market milk protein products have made major contributions in the development of new food products (Mortensen, 1985). In the industrialized nations approximately one-quarter of total dietary proteins is accounted for through utilization of milk proteins (Modler *et al*, 1987). The dairy industry is targeting this market through the development of new and modified dairy ingredients and components such as casein, casein derivatives (Na-, Ca-, K-, caseinates), protein hydrolysates, casein fractions, whey protein concentrate, milk protein concentrate, lactose and fat derivatives.

Traditionally caseinates and whey protein concentrates have been an important source of functional food ingredients. Total milk protein concentrates and/or individual milk protein fractions can be produced by the application of various techniques and technological operations such as precipitation, co-precipitation, separation by ion exchange, centrifugation or membrane processing. For example, milk proteins co-precipitates are produced from skim milk that is heated to 85°C and then the proteins are precipitated by the addition of acids or salts (Kirkpatrick et al ,1985). However, the formation of complex interaction of whey proteins, mainly β-lg, with caseins results in co-precipitates with a lower protein solubility.

To standardize the nomenclature of various milk protein preparations, the International Dairy Federation (IDF) (Lankveld,1988) recommends using the terms "milk protein concentrate" (MPC) and "milk protein" (MP) for milk products containing from 50 to 85% and above 85% protein, respectively.

The proposed definition by IDF is as follows:

> "MPC with defined functional properties is a preserved "milk protein product" which is produced from partially skimmed milk or skim milk by heat treatment (HTST, UHT), by partial removal of lactose and minerals, by protein concentration carried out with membrane separation (UF, sometimes with added diafiltration) and by water removal by evaporation (EV), spray drying(SD) or freeze drying (FD). It contains the casein and the whey protein in their original proportions" Novak, (1991).

Several methods have been proposed for the production of milk protein concentrates. For example, Connolly (1983) reported a process to isolate casein and whey proteins by promoting the interaction between them by alkaline and acidic treatment and the final precipitation of the protein complex at the isoelectric point of casein at pH 4.6. Some processes such as thermal co-precipitation will result in whey proteins denaturation that will affect functional properties of the final product and its industrial application in the food system.

The development and application of the membrane processing technology (ultrafiltration, reverse osmosis, diafiltration, nanofiltration) at the industrial scale resulted in the manufacture of new milk protein products (Glover, 1985). Ultrafiltration may be used to concentrate milk proteins and partial removal of lactose and minerals (Srilaorkul, *et al* 1989). New milk protein concentrate with targeted content of protein and/or lactose

can then be manufactured by drying the UF retentate. The fundamental difference between traditional skim milk powder and UF milk protein concentrate powder is the content of protein and lactose. Furthermore, the application of diafiltration operation to milk retentate may remove remaining lactose and produce UF milk powders containing more than 85% of milk protein.

Milk protein concentrates are used in confectionery and bakery products, meat products, soups and sauces. Each industrial application of milk protein requires specific functional properties that are governed by their composition and physical-chemical characteristics. For example, there is a great market potential for milk protein concentrates with low lactose content in diets for a lactose intolerant population.

Different food products require milk-based ingredients with a very specific composition and targeted functional properties. Each dairy component and manufactured ingredient has its own individual characteristics and properties. The testing and evaluation of functional and physical properties of these products are essential for their final application, utilization and marketing. The functional properties of any manufactured component are affected by many environmental factors and technological parameters during processing of the target product. Therefore, it is important to determine the functional properties of the individual ingredient in a simulated model and then in the applied food systems. Functional properties of milk proteins include solubility, gelation, emulsifying, foaming, heat stability and water binding capacity. These properties are important when applied in food systems such as beverages, bakery, confectionery, frozen

desserts, imitation dairy products, infant formulae, reformed meat, retort stable sauces, salad dressings and yoghurt.

The objective of this study was to manufacture low and high heated milk protein concentrate powders with different levels of protein and lactose by ultrafiltration and/or diafiltration and to study their chemical composition and properties.

2.2. Materials and Methods

2.2.1. Preparation of Ultrafiltered/Diafiltered Skim Milk Retentates

Milk protein concentrates (MPC) were manufactured from commercial HTST milk as illustrated in (Figure 2.1). Pasteurized skim milk (71.6°C/16 sec) and high heated milk (90°C/5 min) was used for the manufacturing of low and high heated UF milk protein concentrate powders. A laboratory DDS 20 unit with twenty 0.72 m^2 membranes with molecular weight cut-off of 20,000 daltons was used for ultrafiltration and/or diafiltration. The skim milk was ultrafiltered and/or diafiltered at 55°C ±1°C, with a transmembrane pressure of 3.5 bar. The extent of the ultrafiltration process was measured by the volumetric concentration factor (VCF) where:

$$VCF = \frac{\text{Volume of original feed}}{\text{Volume of final concentrate}} \quad (2.1)$$

The diafiltration of milk retentates was carried out using water to remove the maximum amount of lactose and to produce MPC with the highest protein levels.

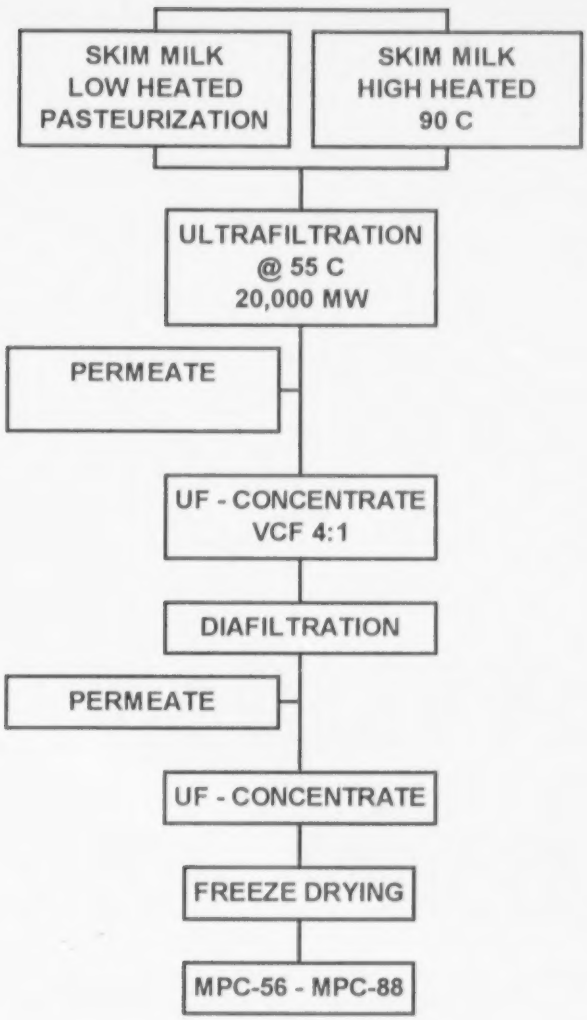

Figure 2.1. **Flow diagram of technology of milk protein concentrate powders manufactured by ultrafiltration and diafiltration.**

Diafiltration was conducted by adding distilled water to the retentate in the ratio 1:1. The diafiltration operation was repeated two, three and four times and samples are referred as DF2, DF3, DF4 and DF4-E. The ultrafiltered and/or diafiltered retentates from all the experiments were freeze dried and stored at 4°C. Part of the skim milk was also freeze dried and kept and used as a control. Six experimental products, in three repetitions, were produced from low temperature heated milk and six products, three repetitions, from high temperature heated milk.

2.2.2. Analytical Methods

2.2.2.1. Composition of Skim Milk and Milk Protein Concentrate Powders Manufactured by Ultrafiltration and/or Diafiltration

Samples of skim milk (control) and MPC powder were analyzed for dry matter, protein, ash, lactose, Ca, P, Mg, Na and K. Dry matter was determined by the hot air oven method (AOAC, 1990). Total protein was determined by the Kjeldahl method using Leco N-Analyzer FP-428 N (AOAC,1990). A factor of 6.38 was applied for N to protein conversion. Ash was determined by igniting samples at 550°C overnight in a muffle furnace. All experimental UF MPC powders were produced in triplicates and chemical analyses were done in duplicate.

2.2.2.2. Determination of Lactose Content in Skim Milk and Milk Protein Concentrate Powders by High Performance Liquid Chromatography

An adapted method from the Food Quality Branch of Alberta Agriculture, Food and Rural Development to determine lactose in skim milk and MPC powders by high performance liquid chromatography (HPLC) was used. HPLC system (Shimadzu) with a

liquid chromatography solvent delivery system LC-6A, sample autoinjector module SIL 6A, system controller SCL-6B, refractive index detector RID-6A and integrator system (C-R3A Chromatopac) was used. Standard carbohydrate solutions were prepared from certified maltose (Fisher Scientific Co.) and alpha lactose (Fisher Scientific Co.). The internal standard solution contained 2.0% (wt/vol) maltose; the lactose standard solution contained 0.2% (wt/vol) each of lactose and maltose. Samples for analysis were prepared by mixing 2 g of MPC powders with 10 ml of internal standard solution and then diluting up to 100 ml with deionized water. Two ml of the reconstituted MPC sample was deproteinized with 1ml of trichloroacetic acid (8% (wt/vol), final concentration) and centrifuged at 1,000x g for 5 min. Deproteinized samples and standard solutions were filtered through 0.45 μm filter (Whatman Ashless filter paper). Lactose was determined by injection of the deproteinized sample onto a Waters Carbohydrate column (3.9x300mm) maintained at 35 °C and separated by isocratic system using an acetonitrile/water (82/18, vol/vol) as a mobile phase at a flow rate 1.6 ml/min. Retention time for lactose was between 11 and 12 minutes depending upon the eluent.

2.2.2.3. Determination of Total Phosphorus Content in Skim Milk and Milk Protein Concentrate Powders.

Skim milk, UF and DF MPC powders (1g) were used for the determination of total phosphorus (IDF, 1990b). Samples were ashed at 550°C for 12 hours and cooled in a desiccator.

Hydrochloric acid (3ml, 1N) was added to silica dishes containing ash and the samples were quantitatively transferred to 100 ml volumetric flasks, filled with deionized

water and shaken to ensure thorough mixing. The solution were filtered through a dry filter (Whatman #42) and 10 ml of the filtrate was pipetted into a 100 ml volumetric flask, diluted to the mark with the deionized water and mixed thoroughly. For determination of phosphorus content 2 ml of the ash solution was transferred into a 50 ml volumetric flask, diluted with 25 ml of deionized water, to which 2 ml of molybdate-ascorbic acid solution was added and then filled to the mark with deionized water. Flasks were heated in a water bath for 15 min. and then cooled to room temperature. Absorbance was measured at 820 nm using a Hewlett-Packard 8452A Diode Array spectrophotometer.

Standard solutions for phosphorus were prepared by diluting 0.439 g of potassium dihydrogen orthophosphate (dried at 105°C overnight) to 1 liter with deionized water. The prepared solution (10 ml) was transferred into a 100 ml one mark volumetric flask and diluted to the mark (1 ml of this standard solution is equal to 10 µg phosphorus). Selected volumes (0 ml, 1 ml, 2 ml, 3 ml and 5 ml) of the standard solution were pipetted into a 50 ml volumetric flask and diluted with 25 ml deionized water and 2 ml of molybdate-ascorbic acid added to each flask and diluted to the mark with deionized water. The flasks were heated in a water bath for 15 minutes, and cooled to room temperature. Absorbance of each of the calibration solutions was measured and the standard curve prepared as illustrated in Figure 2.2.

2.2.2.4. Determination of Calcium and Magnesium Content of Skim Milk and Milk Protein Concentrate Powders.

Calcium [Ca] and magnesium [Mg] content was determined by the flame atomic absorption spectrophotmetric method (IDF, 1992c) using a Perkin-Elmer Atomic

Absorption Spectrophotometer Model 4000 (Perkin-Elmer Corporation, Norwalk, CT) with the following modifications. Stock standard solutions for calcium were prepared by dissolving 1,2481 g of calcium carbonate ($CaC0_3$) in 15 ml of hydrochloric acid solution (4M) diluted up to 1000 ml with deionized water. Twenty ml of the calcium stock solution were transferred into a 500 ml volumetric flask and diluted to the mark with deionized water. Six 100 ml standard solutions were prepared from this solution each one containing 0.27% (wt/vol) lanthanum chloride solution. One milliliter of these standard solutions contained 0 µg, 1µg, 2µg, 3 µg, 4 µg and 5 µg calcium, respectively. Stock standard solution for magnesium, prepared and standardized by McGaw Supply Ltd. (Canlab), was used in the preparation of sequence dilutions 0.1,0.2.0.3, 0.4 p.p.m. The calibration graph for Ca, as illustrated in Figure 2.3., was prepared based on the readings obtained from analysis of standard solutions in the linear range below 0.4 p.p.m (0.2 AB units). The calibration graph for Mg (Figure 2.4.) was prepared based on the readings obtained from analysis of standard solutions in the linear range below 0.4 p.p.m. (0.2 AB units)

The test solutions were prepared by dry ashing 1g of retentates, and skim milk powder. The ash was dissolved in 1ml of nitric acid 25% (m/m) solution and transferred into 250 ml volumetric flask then diluted with deionized water to the mark. Aliquots of

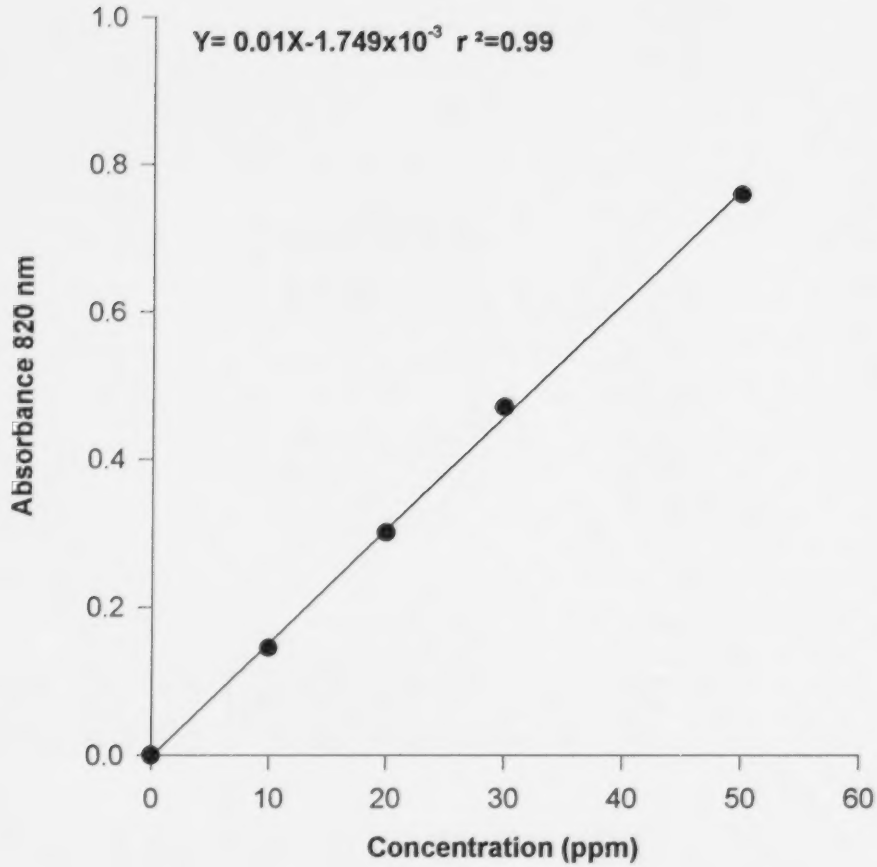

Figure 2.2. Calibration curve for phosphorus (absorbance units vs phosphorus concentration) determined using the spectrophotometric method.

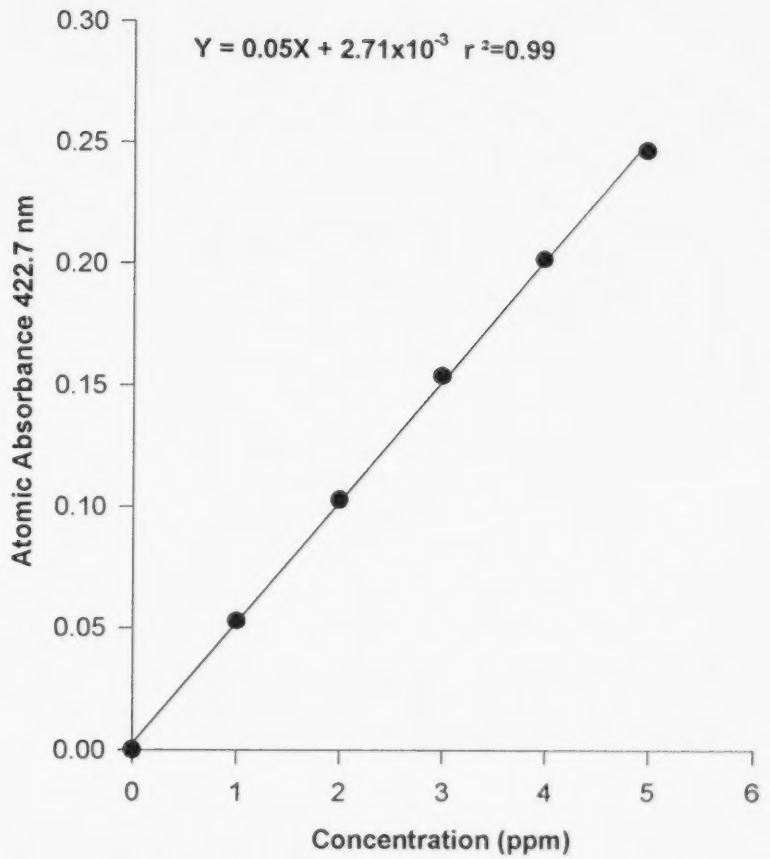

Figure 2.3. Calibration curve for calcium (absorbance units vs calcium concentration) determined by the flame atomic absorption photometric method.

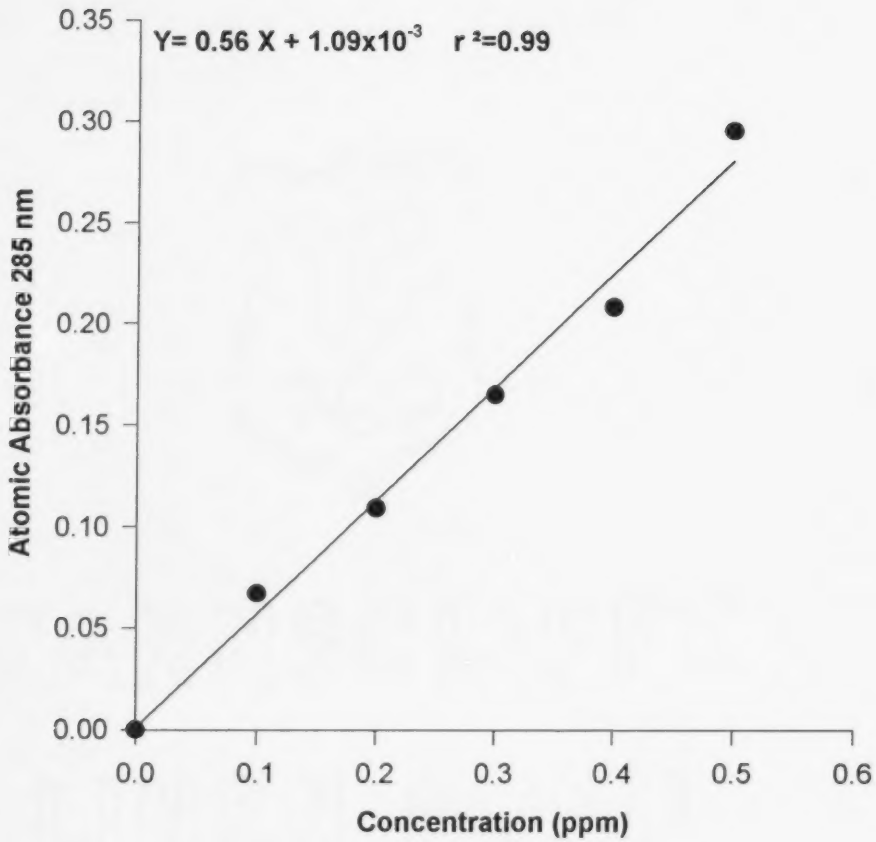

Figure 2.4. **Standard curve of magnesium (absorbance vs magnesium concentration) determined using the flame atomic absorption photometric method.**

5 ml were then removed from these diluted samples and pipetted into a 100 ml volumetric flask to which 10 ml of lanthanum chloride was added and brought up to volume with deionized water. Diluted samples were aspirated directly into the atomic absorption spectrophotometer. A hollow cathode specific for each mineral determination was used.

2.2.2.5. Determination of Sodium and Potassium in Skim Milk and Milk Protein Concentrate Powders.

Total sodium [Na_t] and potassium [K_t] concentrations were determined by flame emission spectrophotometry using a Perking-Elmer 4000 Atomic Absorption Spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT). Samples were prepared according to the IDF (1987a).

2.2.3. Determination of Whey Protein Denaturation by High Performance Liquid Chromatography

Whey protein denaturation was measured by the high performance liquid chromatography (HPLC) method (Ozimek, 1993) using an ion exchange chromatography column (Mono Q HR 5/5). The HPLC system consisted of a binary system (Shimadzu) with two liquid chromatography pumps LC-6A, autoinjector SIL-6A, system controller SCL-6A, UV detector (TosoHaas TSK 6040 UV-VIS) and integrator system (C-R6A Chromatopac). HPLC grade chemicals and deionized water were used throughout the analysis. The buffer system consisted of:

Buffer A: 20 mM tris-HCl, pH 7.0 and
Buffer B: 20 mM tris-HCl, 1M NaCl, pH 7.0.

Buffers used for HPLC analysis were further filtered and deareted through a 0.2 μm Millipore filter. Skim milk and UF MPC solutions (3.2% protein, wt/vol) were diluted

with deionized water and mixed. Casein was precipitated from 10 ml protein solution by adding 0.6 ml of acetate buffer (2.78 M acetic acid and 1.67 M Na acetate) and centrifuged at 100,000 x g (McLean et al., 1984). Supernatant that contained whey proteins was filtered through a 0.22 μm filter prior to injection into a column. Whey protein standards (β-lactoglobulin genetic variants A and B and α-la), in powder form, were dissolved in buffer A and filtered before injection. The calibration curves for β-lg genetic variants A and B and α-la were prepared as a relationship between the total integrated peak area and the volume of sample injected, Figure 2.5.. Sample injection volume was 20 μl and the proteins eluted, at the flow rate of 1ml/min, were measured at 280 nm using a UV detector.

2.2.4. Statistical Methods

Data was analyzed using analysis of variance ANOVA using SAS (version 6) statistical package. Results were expressed as means of three replicates or as otherwise indicated. If and *F* test was significant, a least significant difference test was used to compare individual means.

2.3. Results and Discussion

2.3.1. Composition of Low and High Temperature Skim Milk and UF Milk Protein Concentrate Powders.

Composition of low and high temperature milk protein concentrate powders produced from ultrafiltered/diafiltered skim milk retentates and dry skim milk (control) are presented in Tables 2.1. and 2.2. The protein and lactose concentrations in dry skim milk (control) were 36 and 58-59 %, respectively (Tables 2.1. and 2.2.). During the progressive

concentration of skim milk by ultrafiltration and diafiltration the content of the milk proteins in low and high heated skim milk retentate increased from 36.1 to 87.9 % and 36.2 to 87.5 %, respectively. The concentration of the low and high heated skim milk to volume concentration factor 4:1 resulted in increased protein content to 61.9 and 65,7 % whereas the lactose decreased to 30.7 and 21.6 % in dry powders, respectively.

Diafiltration was introduced in order to produce milk protein concentrates with minimum levels of lactose and maximum levels of proteins. During diafiltration of four times concentrated retentates, the concentration of lactose in low and high heated milk protein concentrates was further decreased to 2.46 and 1.23 %, respectively (Tables 2.1. and 2.2.). It has been demonstrated that the application of ultrafiltration and diafiltration technology facilitates the production of new dairy products that may be designed for specific protein and lactose levels. The degree of lactose removal from milk is affected by the volume concentration factor of retentates and number of diafiltrations (Walstra et al., 1984). Diafiltration of retentates (VCF 4:1) was carried out by mixing equal volumes of retentate and water and then concentrated by ultrafiltration to its former volume. When the process of diafiltration of retentates was repeated two, three and four times the final dry protein concentrates are referred to as DF2, DF3, and DF4, respectively. The fundamental technological purpose of applying multiple diafiltration was to evaluate the relationship between diafiltration and the degree of lactose removed. This is evident in Figure 2.6, which illustrates the distribution of lactose and protein during the combined process of ultrafiltration (concentration factor 4:1) and four diafiltrations.

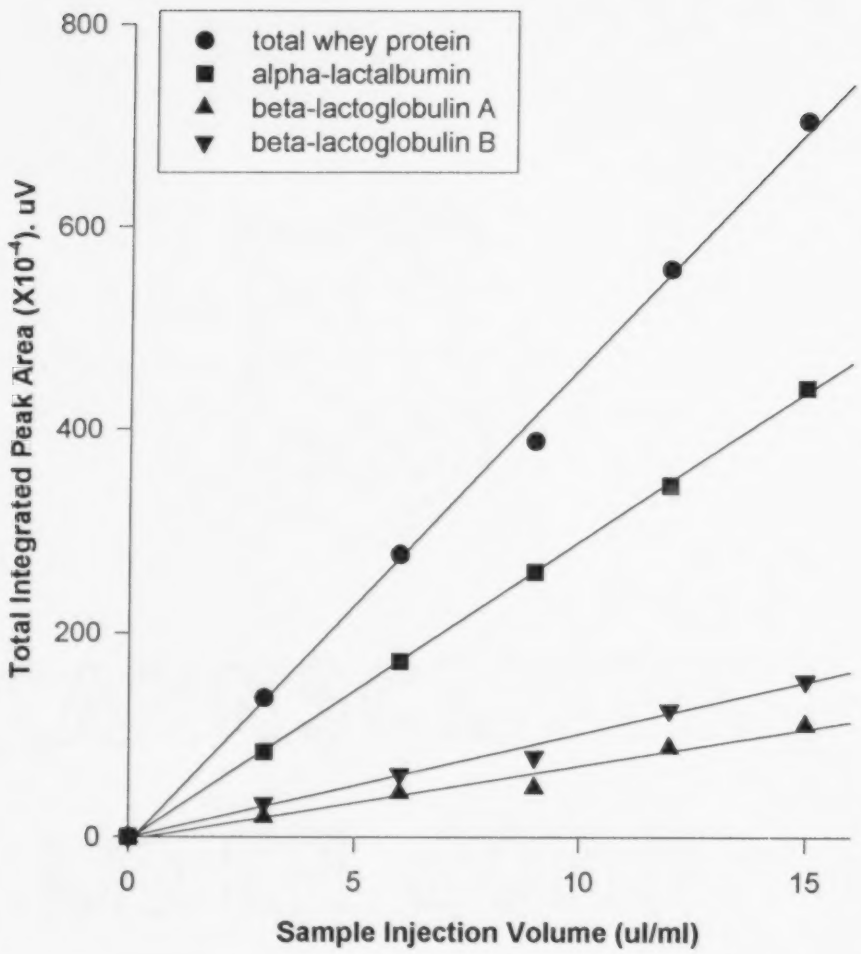

Figure 2.5. **Calibration curves for β-lg variant A and B and α-la determined with High Performance Liquid Chromatography.**

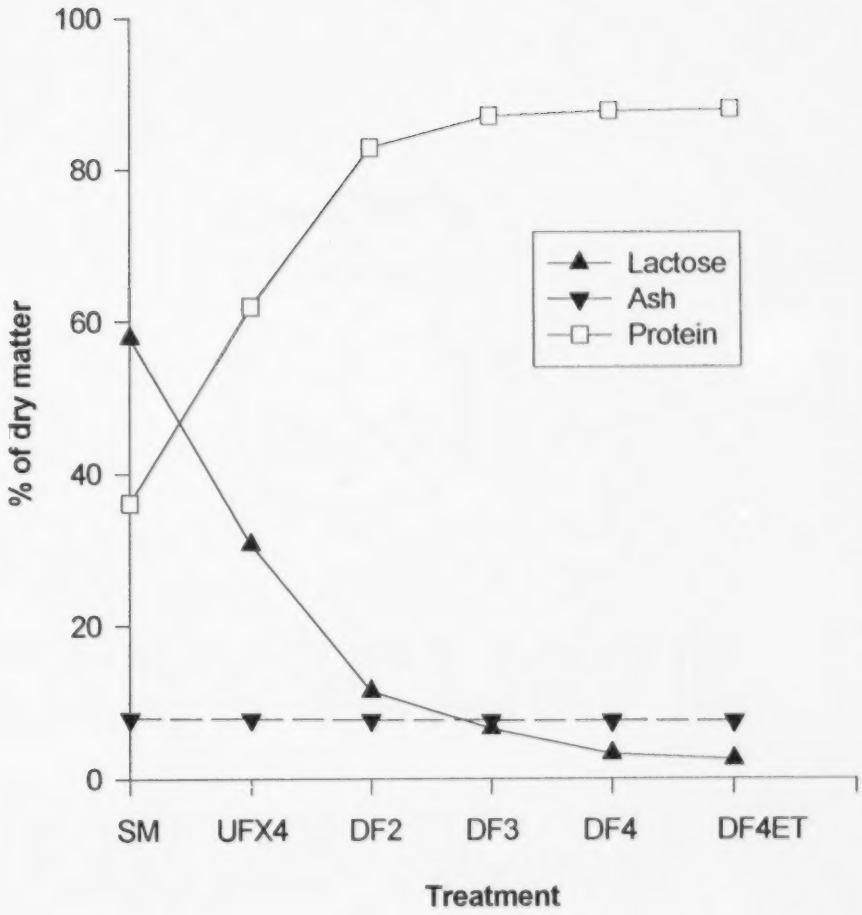

Figure 2.6. **Changes in protein, lactose and ash content as a function of milk processing by ultrafiltration.**

Differences ($P< 0.001$) were found between lactose level in low and high heated dry skim milk and all milk protein concentrates (Tables 2.1. and 2.2.). The protein concentration in low and high heated skim milk increased ($P<0.001$) during ultrafiltration (VCF 4:1) and second diafiltration but not during the second, thirdand fourth diafiltrations (Table 2.1. and 2.2.). There were differences ($P < 0.001$) in high heated skim milk and milk protein concentrate between protein concentrations (VCF 4:1) and second and third diafiltration (DF2 and DF3) as indicated in Tables 2.1 and 2.2. However, further diafiltration (DF4 and DF4-E) did not increase protein concentration. The protein content of the final product milk protein concentrates can be adjusted in a wide range from 36 to 87 % by choosing the degree of ultrafiltration and diafiltration as illustrated in Tables 2.1. and 2.2.

The changes of individual mineral concentration on dry basis (mg/g of total protein) during ultrafiltration and diafiltration are presented in Table 2.3. Calcium decreased ($P<0.05$) during ultrafiltration of skim milk but there was no change during diafiltration. Four fold ultrafiltration and second diafiltration caused decrease ($P<0.001$) in the concentration of sodium and potassium compared to the control. Phosphorus and magnesium decreased ($P<0.001$) during ultrafiltration of skim milk and second diafiltration of retentate. Further diafiltration did not affect the proportions of calcium, phosphorus and magnesium. The majority of calcium is bound to casein in the form of protein-calcium (magnesium)-phosphate complex. Figure 2.7. illustrates changes in concentration of minerals on a dry basis. During ultrafiltration and diafiltration 93.0 % potassium and 88.2 % sodium were removed from milk protein concentrates. Calcium, phosphorus and magnesium decreased by 23.9, 46.7 and 63.4 %, respectively. Srilaorkul *et al.* (1989) indicated that soluble calcium was mostly removed from milk during two fold

Table 2.1. Chemical composition of low temperature skim milk (control) and milk protein concentrate powders manufactured by ultrafiltration and/or diafiltration.

Treatment	Total solids (%)[1]	Total protein (%)[1]	Lactose (%)[1]	Ash (%)[1]
SM[2]	95.3 ± 0.002	36.1 ± 0.019[c]	57.9 ± 0.665[a]	7.87 ± 0.51[a]
UF[3]	95.4 ± 0.007	61.9 ± 0.037[b]	30.7 ± 0.621[b]	7.99 ± 0.50[a]
DF2[4]	95.4 ± 0.002	82.8 ± 0.023[a]	11.4 ± 0.384[c]	7.72 ± 0.51[a]
DF3	96.0 ± 0.002	86.9 ± 0.015[a]	6.56 ± 0.457[d]	7.63 ± 0.51[a]
DF4	94.8 ± 0.016	87.7 ± 0.005[a]	3.20 ± 0.491[e]	7.60 ± 0.50[a]
DF4-E[5]	95.3 ± 0.019	87.9 ± 0.006[a]	2.46 ± 0.173[e]	7.51 ± 0.52[a]

[a,b,c,d,e] Means not sharing the same superscript are different. ($P < 0.001$) whithin each column

[1] Each number is mean of three replicates and the protein, lactose and ash are expressed as percent of total solids ± standard deviation

[2] SM = skim milk powder (control)

[3] UF = ultrafiltration 4:1

[4] DF2, DF3 and DF4 = Second, third and fourth diafiltration, respectively.

[5] DF4-E = Fourth diafiltration with ethanol.

Table 2.2. Chemical composition of high temperature skim milk (control) and milk protein concentrate powders manufactured by ultrafiltration and/or diafiltration.

	Total solids (%)[1]	Total protein (%)[1]	Lactose (%)[1]	Ash (%)[1]
SM[2]	95.2 ± 0.0080	36.2 ± 0.0014[d]	59.3 ± 0.52[a]	9.10 ± 0.0009[a]
UF[3]	95.7 ± 0.0055	65.6 ± 0.0052[c]	21.6 ± 0.19[b]	8.20 ± 0.0012[b]
DF2[4]	95.7 ± 0.0036	83.2 ± 0.0015[b]	9.63 ± 0.38[c]	7.88 ± 0.0001[c]
DF3	95.8 ± 0.0011	86.0 ± 0.0037[a]	4.85 ± 0.42[d]	7.83 ± 0.0015[c]
DF4	95.4 ± 0.0064	87.7 ± 0.0108[a]	2.16 ± 0.31[e]	7.74 ± 0.0006[c]
DF4-E[5]	96.0 ± 0.0130	87.5 ± 0.0019[a]	1.22 ± 0.23[e]	7.74 ± 0.0005[c]

[a,b,c,d,e] Means not sharing the same superscript are different. ($P < 0.001$) within each column

[1] Each number is mean of three replicates and the protein, lactose and ash are expressed as percent of total solids ± standard deviation

[2] SM = skim milk powder (control)

[3] UF = ultrafiltration 4:1

[4] DF2, DF3 and DF4 = Second, third and fourth diafiltration, respectively.

[5] DF4-E = Fourth diafiltration with ethanol.

Table 2.3. The content of mineral in low heated skim milk (control) and milk protein concentrate powders manufactured by ultrafiltration and/or diafiltration

	Minerals content (mg/g protein)[1]				
Treatment	Ca	P	K	Na	Mg
SM[2]	35.3 ± 0.20^{a}	29.0 ± 2.60^{a}	39.4 ± 2.54^{a}	12.9 ± 0.40^{a}	3.20 ± 0.07^{a}
UF[3]	30.4 ± 2.39^{ab*}	20.9 ± 2.07^{b***}	15.3 ± 2.62^{b***}	5.85 ± 0.30^{b***}	1.86 ± 0.19^{b***}
DF2[4]	29.4 ± 0.68^{b*}	17.3 ± 0.73^{bc***}	5.33 ± 0.55^{c***}	2.70 ± 0.07^{c***}	1.41 ± 0.03^{bc***}
DF3	27.4 ± 2.20^{b}	16.4 ± 0.66^{c}	3.72 ± 0.38^{c}	2.03 ± 0.08^{cd***}	1.24 ± 0.12^{c}
DF4	27.9 ± 1.85^{b}	16.9 ± 0.86^{c}	3.01 ± 0.18^{c}	1.69 ± 0.16^{d}	1.20 ± 0.15^{c}
DF4[5]	26.9 ± 5.16^{b}	15.5 ± 0.75^{c}	2.76 ± 0.09^{c}	1.51 ± 0.05^{d}	1.17 ± 0.17^{c}

^{a,b,c,d,e} Means not sharing the same superscript are different. *($P<0.05$), ***($P<0.001$) within each column

[1] Each number is mean of three replicates ± standard deviation

[2] SM = skim milk powder (control)

[3] UF = ultrafiltration 4:1

[4] DF2, DF3 and DF4 = Second, third and fourth diafiltration, respectively. [5] DF4-E = Fourth diafiltration with ethanol.

ultrafiltration while the removal of soluble phosphorus and magnesium required three fold concentration. More calcium can be removed by changing the pH of milk before or during ultrafiltration /diafiltration. (Babella, 1989)

2.3.2. Heat Classification of Low and High Temperature UF Milk Protein Concentrate Powders.

The classification of skim milk powders into low, medium and high heated is based on the degree of whey proteins denaturation. The degree of whey protein denaturation will depend on the temperature and time to which milk is subjected to heat. The extent to which whey proteins are denatured in milk powder will define its application in the dairy and non dairy industry. For example, milk powder that is designed for cheese making should be low heated and contain not less than 6 mg of undenatured whey protein nitrogen per gram of powder. The justification for requirement of low heat skim milk powder for cheesemaking is based on the need for good milk coagulation properties when clotting enzymes are added. High temperature, above 85-90°C, causes whey protein denaturation, mainly β-lactoglobulin and formation of complexes with κ-casein in casein micelles in milk. The coverage of casein micelles with denatured whey proteins slows down the access of clotting enzymes (ie. chymosin) to the κ-casein peptide bond 105-106 that is the foundation of converting milk into cheese (Kilara and Sharkasi, 1986).

Undenatured whey protein (UWP) values for low and high heated skim milk and milk protein concentrate powders manufactured by ultrafiltration and diafiltration, expressed as mg of undenatured sum of (β-lg A and B and α-la) per gram of protein are

indicated in Table 2.4. Differences ($P<0.001$) between the content of UWP in low heated and high heated skim milk and protein concentrate powders were found.

In this study, the average concentration of UWP in low and high heated milk protein concentrate powders were 136 and 4.40 mg per g of total protein, respectively (Table 2.4.).

Traditional classification of skim milk powders into low, medium and high heated is shown in Table 2.4. (Jensen, 1988). Current heat classification of milk powder is based on the measurement of undenatured whey protein nitrogen (UWPN) and is expressed in milligrams of UWPN per gram of powder. This manner of expression does not allow comparison of results obtained for milk protein concentrate powders in this study. Therefore, we would like to propose the classification of milk protein concentrate powders into low, medium and high heated based on concentration of undenatured sum of (β-lg A and B and α-la) and to express the results in milligrams of UWP per gram of total milk proteins.

The justification of the proposed classification is based on the fact that traditional milk powders do not differ much in gross composition including the content of whey proteins. In contrast, milk protein concentrate powders produced from ultrafiltered and diafiltered retentates are characterized by significant differences in protein concentration (Table 2.1. and 2.2.) Therefore the content of whey proteins, denatured or undenatured, can not be compared to traditional skim milk powders. For example, low heat skim milk powder should contain more than six milligrams of UWPN per gram of total powder that

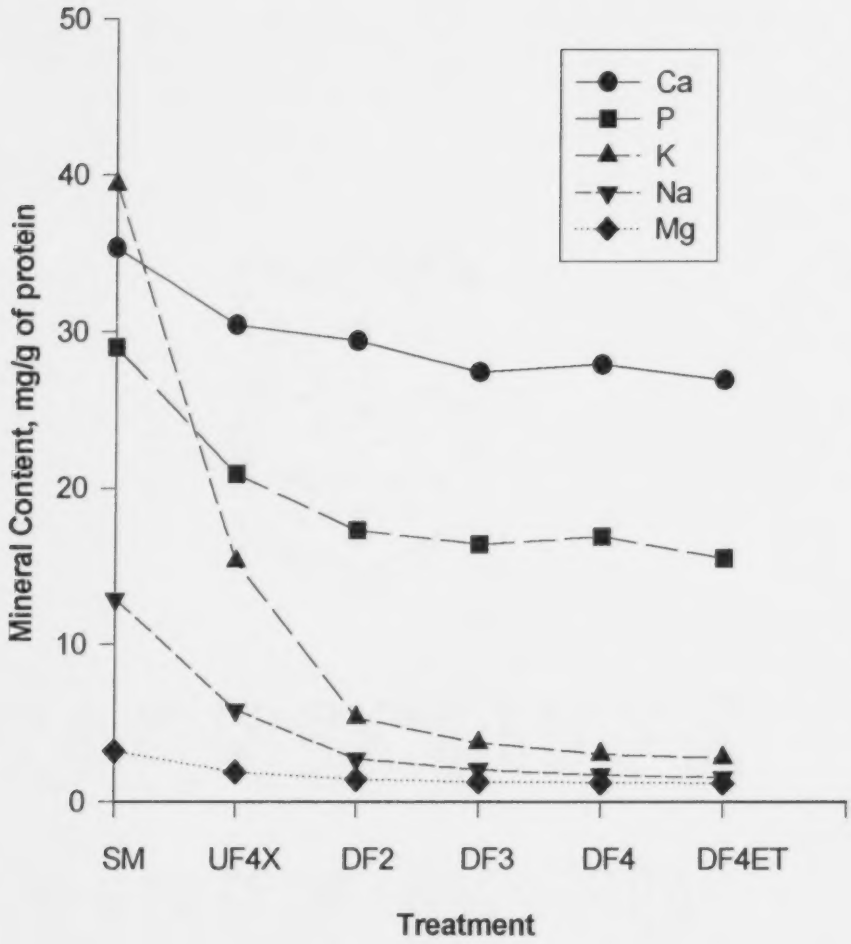

Figure 2.7. **Changes in the mineral concentration as a function of the milk processing by ultrafiltration and diafiltration.**

Table 2.4. Heat classification of skim milk and milk protein concentrate powders.

	LT (72.6°C/16 sec)	HT (90°C/5 min)
	mg UWP (α-la+βlg)/g TMP[1]	
SM[2]	133.6[a]	4.14[b]
UF[3]	132.3[a]	6.10[b]
DF2[4]	130.8[a]	3.26[b]
DF3	143.6[a]	4.30[b]
DF4	139.7[a]	4.21[b]
DF4- E[5]	131.6[a]	2.76[b]
Average	136	4.40

[a,b] Means not sharing the same superscript are different. ($P < 0.001$)

[1] Each number is mean of two replicates UWP = undenatured whey proteins, TMP = total milk protein

[2] SM = skim milk powder (control)

[3] UF = ultrafiltration 4:1

[4] DF2, DF3 and DF4 = Second, third and fourth diafiltration, respectively.

[5] DF4-E = Fourth diafiltration with ethanol.

Table 2.5. Heat classification of skim milk powder.

Classification : Whey protein nitrogen (WPN)/g of powder		
Low heat	WPN/g powder not less than	6.0
Medium heat	WPN/g powder from	1.51-5.99
High heat mg	WPN/g powder not more than	1.5

Adapted from Jensen, 1988.

may correspond to not less than 109 mg UWP per gram of total protein. Furthermore, the high heated skim milk powder having not more than 1.5 mg UWPN per gram of powder can also be expressed as product having not more than 27 mg of UWP per gram of total protein. The degree of whey protein denaturation in milk protein concentrate powders will affect their functional properties and their application there is a need for introduction and implementation of MPC into low medium and high heated classes. This will require more in depth studies on the comparative methods of heat denaturation measurement, evaluation and standardization.

2.4. Conclusions

Ultrafiltration combined with diafiltration is an effective method to producing milk protein concentrates from skim milk.

The most attractive feature of ultrafiltration is its flexibility in controlling protein content to any desired value between 35% in traditional skim milk powder and 87 % in milk protein concentrate powders. This new technology allows the manufacture of milk protein concentrates that can be tailored to produce a desired composition for targeted applications.

In this study, low and high heated milk protein concentrate powders containing from 50 to 87 % protein were manufactured by membrane processing. Lactose concentration decreased from 58.1 to 2.55 % as the protein increased from 35.6 to 87.6 % .

It is important to remember that the final concentration of individual components (lactose, minerals and protein) in milk protein concentrates is affected by membrane

processing and particularly by concentration factor, diafiltration, pH and/or any other technological and environmental modifications.

There is a good export opportunity for MPC for the nutritional application for populations were lactose malabsorption is widely prevalent.

The content of undenatured whey protein (UWP), mg/g of milk protein, should be used as an index for classification of MPC into low, medium and high temperature treated.

It is proposed that powders containing various protein levels should be classified into heat treatment classes based on the content of UWP.

Low and high heated milk protein concentrates were manufactured containing 136 and 4.40 mg UWP per g of total protein, respectively.

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Chapter 3

3. Water Sorption Isotherm and Surface Hydrophobicity of Milk Protein Concentrate Powders

3.1. Introduction

Ultrafiltration is a process technology used in the concentration and selective fractionation of milk components. Membrane technology such as ultrafiltration has undergone many advances and is used in the dairy industry in several commercial applications (Jimenez-Flores et al 1986, Srilaorkul et al, 1989). Powders with a protein content between 50-85% manufactured by membrane separation and/or diafiltration are refered to as milk protein concentrates (MPC), while powders with a protein content over 85% are refered to as milk proteins (MP) (Lankveld,1988).

Milk proteins possess an array of functional properties, and as such are an important class of functional ingredients in dairy products and non dairy products. Functional characteristics of milk proteins such as foaming capacity, emulsifying capacity and moisture sorption, among others, depend considerably on the state of the protein (Damodaran, 1994). Li-Chan and Nakai (1989) concluded that functional properties of proteins are affected by hydrophobic, electrical and stearic properties. Modification of proteins due to technological and processing methods such as precipitation and heat treatment affect the hydrophobic interactions. Kato (1991) reported that preheating of fluid skim milk alters the casein micelle surface hence affecting the hydrophobic properties of resulting non-fat dry milk powders. Hydrophobic interactions are largely responsible

for the reactivity of proteins in relation to other macromolecules in solution (Kinsella *et al.*, 1994; Ozimek *et al.*, 1994; Kato and Nakai, 1980).

Hydrophobic sites near the surface of macromolecular bodies such as casein micelles, proteins and polymers may be studied by taking advantage of the modification of their properties by suitable spectroscopic markers (Kinsella et al, 1981).

There are a different methods to quantify the surface hydrophobicity (S_o) of milk proteins (Lieske and Konrad, 1994). Hydrophobic affinity chromatography was used by Keshavarz and Nakai (1979) to determine the effective hydrophobicity. Keshavarz and Nakai (1979) also estimated hydrophobic sites using the hydrophobic partition method. Hydrophobic interaction chromatography was used by Kato (1991) in their study on the effect of preheating temperature on the hydrophobic properties of milk proteins. Alternate methods using hydrophobic probes employing 8-anilino-1-naphthalene sulfonate (ANS) or cis-parinaric acid (CPA) as probes to observe fluorescence intensity have also been developed to detect S_o of the native proteins in solution (Kato *et al.*, 1980 and Sklar *et al.*, 1977). Hydrophobic probe methods using 8-anilino-1-naphthalene sulfonate (ANS) seems an adequate probe to determine hydrophobicity (Nakai *et al*, 1988 and Nakai *et al* 1989). The nonpolar rings found in ANS naturally aggregate with the hydrophobic sites on the surface of the milk protein. The number of bound ANS molecules increase with increasing concentration of ANS until a saturation limit is reached. ANS is known for its tendency to project a fluorescent emission when excited by light in the 370-480 nm range. Therefore, as the number of ANS molecules bound to the hydrophobic sites expand, the fluorescence emission intensifies. A comparison between the fluorescence intensities observed in many proteins using the same concentration of ANS defines an index for estimating the

hydrophobicity (Slavik, 1982). Cis-Parinaric acid has also been used to spectroscopically characterize the S_o of certain proteins (Sklar *et al.*, 1977), although it is much more difficult to work with than ANS. Estimating the S_o of dairy proteins using ANS remains the most comprehensive and effective method.

The physical, chemical, quality, and functional characteristics of food components are influenced by the content and physical state of water. Via electrostatic, hydrophobic and Van der Waals interactions and thiol oxidation irreversible conformational changes can occur at critical moisture levels. Protein stability of stored powders can be affected by the water content and activity and fluctuations in moisture and temperature. For example, an irreversible transit from ion to the stable crystalline state of metastable amorphous lactose is caused by exposing milk powder to stressful conditions, such as high relative humidiy (RH) and/or increased temperatures. This results in reduction of the solubility of the dried milk and lumping and caking of the powder.(Berlin *et al.*, 1970; Vuataz, 1988).

Berlin et al (1968) observed that at increasing water activities (A_w) components of milk powder adsorbed moisture progressively. Moisture sorption of proteins occurs at lower A_w in the order of A_w: casein (0.01 - 0.30) > whey proteins (0.05 to 0.35) > lactose (0.2 to 0.6) and milk salts (0.6 to 1.0); the A_w values in parentheses indicate a range of maximum water sorption. Gregg et al (1981) states that freeze dried samples of whey concentrates, when observed by electron microscopy, had an open porous structure with many voids, while the spray dried samples had a large surface to volume ratio due to a large number of porous, small spheres.

The understanding of sorption behaviour of powders is important for the evaluation of water uptake, porosity and sorption/desorption enthalpies. Interactions

between water and food substances can be studied by the determination of the moisture content, sorption behaviour and A_w. Moisture sorption isotherms provide useful information for food processing operations such as drying, mixing, packaging, and storage. (Labuza, 1982a). The growth of microorganisms in food products can be controlled having information on water sorption behaviour and factors affecting water activity (Le Maguer, 1987).

The objective of this study was to determine the water sorption isotherm and surface hydrophobicity properties of milk protein concentrate powders produced from ultrafiltered and diafiltered retentates in relation to their composition.

3.2. Materials and Methods

3.2.1. Preparation of Ultrafiltered/Diafiltered Skim Milk Retentates

Skim milk heat treated at 71,6°C/ 15s and 90°C/5 min prior to ultrafiltration was ultrafiltered (UF) and diafiltered at 50°C in an ultrafiltration unit (LAB-Module DDS-20). For separation and concentration twenty membranes (0.0018 m^2 GR 60-PP) with molecular weight cut-off of 20,000 daltons were used. A transmembrane pressure of 3.5 bar was used during UF and DF. A final volume concentration factor (VCF) 4:1 was used to concentrate the skim milk (UF), where VCF is the ratio of the volume of the original feed to the final volume of the retentate. Part of the skim milk (SM) was kept and used as a control (CF 1:1). Diafiltration was conducted by adding distilled water to the retentate in the ratio 1:1, to remove the maximum amount of lactose and to produce milk protein concentrate (MPC) powders with the highest protein levels. The diafiltration operation was repeated two, three and four times and samples are referred as DF2, DF3, DF4 and

DF4-E. Samples of retentates were taken after ultrafiltration and second, third and fourth diafiltration. Skim milk (control) and the ultrafiltered and/or diafiltered retentates were freeze dried and kept at 4°C. Six experimental products, in three repetitions, were produced from low temperature heated milk and six products, three repetitions, from high temperature heated milk.

3.2.2. Analytical Methods

All chemical analyses were carried out in duplicate for dry matter, protein, ash, lactose, Ca, P, Mg, Na and K. Total protein of MPC and of skim milk (control) powder was determined by the Kjeldahl method (AOAC,1990) using Leco N-Analyzer FP-428 N determinator. A factor of 6.38 was applied for N to protein conversion. Dry matter was determined by the hot air oven method (AOAC, 1990). To determine ash content the crucibles were ignited overnight at 550°C in a muffle furnace, cooled and weighed.

High performance liquid chromatography (HPLC) was used to determine the lactose content of milk protein concentrates and skim milk (control) powders. The method was adapted from the Food Quality Branch of Alberta Agriculture, Food and Rural Development. The HPLC system consisted of a Shimadzu with a liquid chromatography solvent delivery system LC-6A, sample autoinjector module SIL 6A, system controller SCL-6B, refractive index detector RID-6A and integrator system (C-R3A Chromatopac). The HPLC was calibrated using a purified standard carbohydrate solution of maltose (Fisher Scientific Co.) and alpha lactose (Fisher Scientific Co.). The internal standard solution contained 2.0 % (wt/vol) maltose; the lactose standard solution contained 0.2% (wt/vol) each of lactose and maltose. Samples were prepared for analysis by mixing 2 g of

MPC and SM powders with 10 ml of internal standard solution and diluting up to 100 ml with deionized water. Two ml of the reconstituted sample was deproteinized with 1 ml of trichloroacetic acid (8% (wt/vol) and centrifuged at 1,000x g for 5 min. Deproteinized samples and standard solutions were filtered through a 0.45 μm filter (Whatman Ashless filter paper). A Waters Carbohydrate column (3.9x300 mm) maintained at 35 °C was used to separate lactose. Acetonitrile-water (82/18, vol/vol) was the mobile phase with a fkiw rate of 1.6 ml/min. Retention time for lactose was between 11 and 12 min depending upon the eluent.

Samples of skim milk and milk protein concentrates powders (1g) were ashed at 550°C for 12 hours. Total phosphorus was determined according to the Internatinal Dairy Federation (IDF) standard method (IDF, 1990b).

Samples and standards were analyzed using a spectrophotometer (Hewlett Packard 8452A Diode Array) at a wavelength of 820 nm.

Calcium [Ca] and magnesium [Mg] content of skim milk and milk protein concentrates powders were determined according to the procedure described in IDF standard method (IDF, 1992c). Calcium and magnesium content of milk protein concentrates and skim milk powders were determined using a Perkin-Elmer atomic Absorption Spectrophotometer model 4000 (Perkin-Elmer Corporation, Norwalk, CT) (IDF,1992c).

Sodium [Na_t], and potassium [K_t] contents of milk protein concentrates and milk protein powders were determined using a Perking-Elmer 4000 Atomic Absorption Spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT). Ashed samples were dissolved in 1 ml nitric acid 25% (m/m) solution and then rinsed in a 100 ml volumetric

flask with deionized water. The spectrometer was calibrated using standards prepared in the range of the ash samples (IDF, 1987a). Samples and standards were analyzed for Na and K at a wavelengths of 589 nm and 766.5 nm respectively.

Whey protein denaturation was measured by (HPLC) method (Ozimek, 1993) on an ion exchange chromatography column (Mono Q HR 5/5). The HPLC system consisted of a binary system (Shimadzu) with two liquid chromatography pumps LC-6A, autoinjector SIL-6A, system controller SCL-6A, UV detector (TosoHaas TSK 6040 UV-VIS) and integrator system (C-R6A Chromatopac). HPLC grade chemicals and deionized water were used throughout analyses. The buffer system consisted of :

Buffer A: 20 mM tris-HCl, pH 7.0 and
Buffer B: 20 mM tris-HCl, 1M NaCl, pH 7.0.

Buffers used for HPLC analysis were further filtered and deareted through a 0.2 μm Millipore filter. Skim milk and MPC solutions (3.2% protein, wt/vol) were diluted with deionized water and mixed. Casein was precipitated from a 10 ml protein solution by adding 0.6 ml of acetate buffer (2.78 M acetic acid and 1.67 M Na acetate) and centrifuged at 100,000 x g (McLean et al., 1984). Supernatant that contained whey proteins was filtered through 0.22 μm filter prior to injection into a column. Whey protein standards (β-lg genetic variant A and B and α-la), in powder form, were dissolved in buffer A and filtered before injection. The calibration curves for β-lg genetic variant A and B and α-la were prepared as a relationship between the total integrated peak area and the volume of sample injected (Figure 3.1). Whey proteins were eluted at the flow rate of 1 ml/min and protein detected by UV detector at 280 nm.

3.2.3. Sorption Isotherms

Binary saturated aqueous salt solutions (LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, NaBr, NaCl, KCl, $BaCl_2$) were prepared according to Spiess *et al.* (1987) in order to provide various constant relative humidities (11 - 90%). Each slurry was placed in a separate desiccator. To determine adsorption isotherm data, the samples of MPC concentrates and SM powder (about 1g) were accurately weighed in aluminum pans and then dried over P_2O_5 (as close to a_w=0) at 30°C in a vacuum oven. Dried samples were kept at 20°C in desiccators which contained saturated salt solutions. Samples were weighed every week until a weight change of <0.002 g was recorded on two consecutive weighings. The equilibrium moisture content (EMC) was obtained after 7-10 weeks of exposition (Labuza, 1985c).

The monolayer moisture contents and thermodynamic constants were determined using the equations of Brunauer, Emmett and Teller's (BET) (Labuza 1984),

$$\frac{a_w}{(1-a_w) * M} = \frac{1}{V_m * c} + \frac{c-1}{V_m * c} * c \qquad [1]$$

The BET surface area was calculated using equation

$$A = V_m * 1/m_h * N * A_h \qquad [2]$$

where, A: - monolayer surface area (m^2/g solids)

A_h: - surface area of one water molecule ($10.6*10^{-20} m^2$)

V_m: - monolayer moisture content (g/g solids)

m_h: - molecular weight of water (18 g/mole)

N: - Avogadro's number ($6.02*10^{23}$ molecules/mole)

3.2.4. Surface Hydrophobicity

Low and high heated skim milk and milk protein concentrate (MPC) powders were solubilized in phosphate buffer at pH 6.8. The resulting solutions had a protein content of 3.1%. Aliquots solutions (100 µl) were diluted with 50 mM potassium phosphate buffer, at pH 6.8, to a final volume of 10 ml. Solutions of low and high heated MPC and skim milk (3 ml) were titrated with 200 µM ANS (8-anilino-1-naphtalene sulphonate) until saturation was achieved. An aqueous solution hydrophobic probe of 200 µM ANS (8-anilino-1-naphtalene sulphonate) (Eastmant-Kodak CO., Rochester, NY) in phosphate buffer pH 6.8 was prepared and used for the ligand binding study. The ANS protein conjugates were excited at λ_{ex}=390 nm and the relative fluorescence intensity was measured at λ_{em}=480 nm in a Perkin Elmer Luminescence Spectrofluorometer (LS-50 B).

The binding data were analyzed according to Closs *et al* (1990) and the surface hydrophobicity, S_0, of low and high heated milk protein concentrate solutions was expressed as:

$$S_0 = FI / P$$

where FI is the fluorescence intensity of the protein-flurophore conjugate, and *P* is the total protein concentrate of the ANS-MPC solution (mg/ml).

3.2.5. Statistical Methods

Data were analyzed using ANOVA using SAS (version 6) statistical package. Results were expressed as means of three replicates or as otherwise indicated. If and *F*

test was significant, a least significant difference test was used to compare individual means.

3.3. Results and Discussions

3.3.1. Chemical Composition of Low and High Heated Milk Protein Concentrate Powders

The data of low and high heated milk protein concentrates (MPC) and skim milk powders showed variations in their composition and thermal characteristics Tables 3.1. and 3.2. show the composition of the low and high heated MPC powders manufactured from skim milk by ultrafiltration and diafiltration. The main differences in the chemical composition of MPC, that may affect water sorption isotherms and surface hydrophobicity are in protein content (36-87%) and lactose (2-59%) (Table 3.1. and 3.2..). Increased protein concentration was observed during the progressive concentration of low and high heated skim milk by ultrafiltration from 36.1 to 87.9% and 36.2 to 87.5%, respectively. Table 3.3. shows the changes of individual mineral concentration on dry matter (mg/g of total protein) during ultrafiltration and diafiltration. K and Na concentration of milk protein concentrates decreased during ultrafiltration and diafiltration by 93.0 and 88.2 %, respectively. Ca, P and Mg also decreased by 23.9, 46.7 and 63.4%, respectively.

3.3.2. The Effect of Low and High Temperature Treatment on Protein Denaturation in Milk Protein Concentrate Powders.

Reliable measurement of whey protein denaturation in MPC was achieved by HPLC. Upon heating milk, β-lactoglobulin is denatured as indicated in Figure 3.1. This enables the monitoring of changes in the chemical and physicochemical properties of whey

proteins during heat treatment. Casein and denatured whey proteins were separated from native whey proteins by precipitation at pH 4.6 and centrifugation. The identification of eluted peaks was established by HPLC elution profile of purified whey protein standards. From the integrated peak area of the whey protein profile, 136 and 4.40 (mg of UWP/ g of protein) was determined for low and high heatd MPC, respectively.

Significant difference ($P<0.001$) between low heated and high heated skim milk and milk protein concentrate powders were found in the concentration of undenatured whey protein (UWP) per gram of total milk proteins and results are shown in Table 3.4.. The degree of denaturation of whey proteins affects the functional properties of proteins and hence, its final application.

3.3.3. Water Sorption Isotherms

Water Sorption isotherms for milk protein concentrates are shown in (Figure 3.2 and 3.3) The isotherms obtained are smooth sigmoid Type II according to the BET classification. In general, equilibrium moisture content (EMC) was similar for all products. although the EMC for skim milk powder was lower than that of milk protein concentrate powders with higher protein concentration.

At higher water activities ($A_w>0.65$) there was a steep increase in moisture concentration for SM and MPC powder. This may be explained by moisture adsorption by salts and/or gradual dissolution of crystalline lactose (Kinsella *et al*, 1986). There was no difference between low and high heated skim milk powder and milk protein concentrates in the EMC.

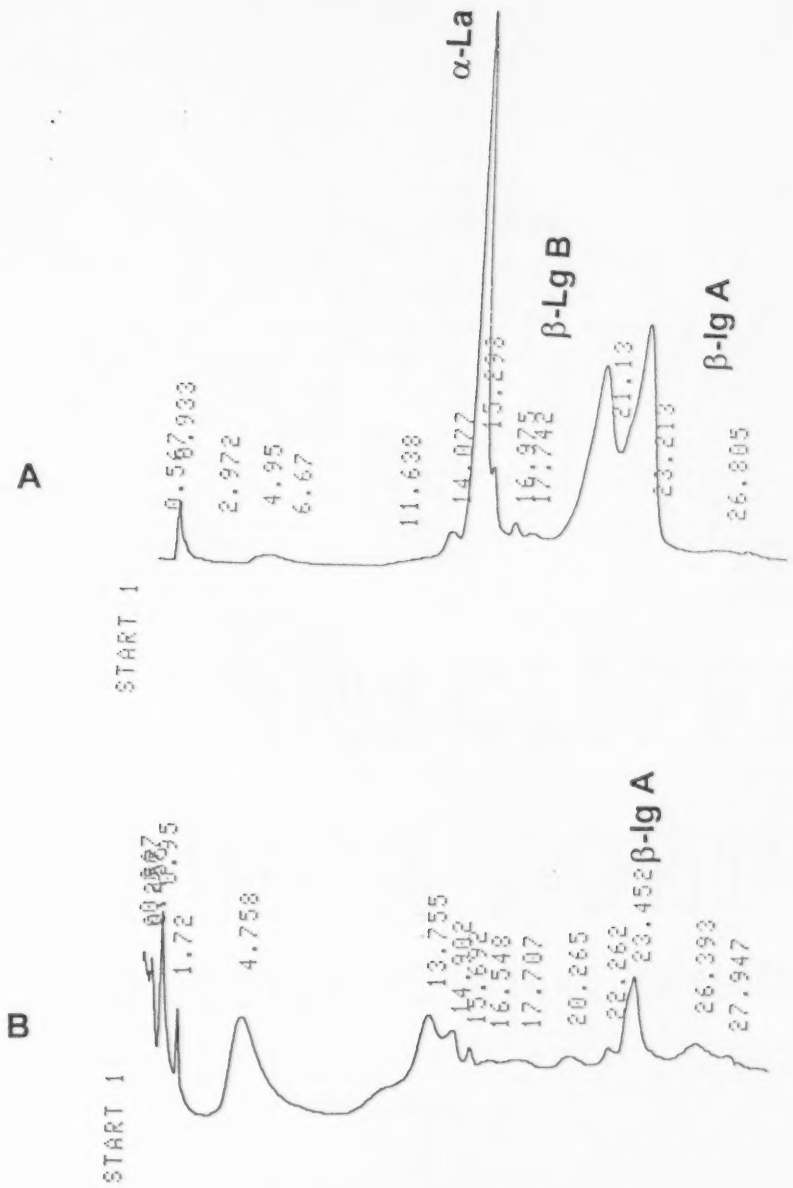

Figure 3.1. **The effect of heat treatment on whey protein denaturation in milk protein concentrate powders measured by IE-HPLC on Mono Q HR 5/5 column. A-** low heated; **B-** 90°C..

Table 3.1. Chemical composition of low temperature milk protein concentrate powders manufactured by ultrafiltration and/or diafiltration.

Treatment	Total solids (%)[1]	Total protein (%)[1]	Lactose (%)[1]	Ash (%)[1]
SM[2]	95.3 ± 0.002	36.1 ± 0.019[c]	57.9 ± 0.665[a]	7.87 ± 0.51[a]
UF[3]	95.4 ± 0.007	61.9 ± 0.037[b]	30.7 ± 0.621[b]	7.99 ± 0.50[a]
DF2[4]	95.4 ± 0.002	82.8 ± 0.023[a]	11.4 ± 0.384[c]	7.72 ± 0.51[a]
DF3	96.0 ± 0.002	87.0 ± 0.015[a]	6.56 ± 0.457[d]	7.63 ± 0.51[a]
DF4	94.9 ± 0.016	87.7 ± 0.005[a]	3.20 ± 0.491[e]	7.60 ± 0.50[a]
DF4-E[5]	95.3 ± 0.019	87.9 ± 0.006[a]	2.46 ± 0.173[e]	7.51 ± 0.52[a]

[a,b,c,d,e] Means not sharing the same superscript are different. ($P < 0.001$) whithin each column

[1] Each number is mean of three replicates, the protein, lactose and ash are expressed as percent of total solids ± standard deviation

[2] SM = skim milk powder (control)

[3] UF = ultrafiltration 4:1

[4] DF2, DF3 and DF4 = Second, third and fourth diafiltration, respectively.

[5] DF4 = Fourth diafiltration with ethanol.

Table 3.2. Chemical composition of high temperature milk protein concentrate powders manufactured by ultrafiltration and/or diafiltration.

	Total solids (%)[1]	Total protein (%)[1]	Lactose (%)[1]	Ash (%)[1]
SM[2]	95.2 ± 0.0080	36.2 ± 0.0014[d]	59.3 ± 0.52[a]	9.10 ± 0.0009[a]
UF[3]	95.8 ± 0.0055	65.6 ± 0.0052[c]	21.7 ± 0.19[b]	8.20 ± 0.0012[b]
DF2[4]	95.7 ± 0.0036	83.2 ± 0.0015[b]	9.63 ± 0.38[c]	7.88 ± 0.0001[c]
DF3	95.8 ± 0.0011	86.1 ± 0.0037[a]	4.85 ± 0.42[d]	7.83 ± 0.0015[c]
DF4	95.4 ± 0.0064	87.8 ± 0.0108[a]	2.16 ± 0.31[e]	7.74 ± 0.0006[c]
DF4-E[5]	96.0 ± 0.0130	87.5 ± 0.0019[a]	1.22 ± 0.23[e]	7.74 ± 0.0005[c]

[a,b,c,d,e] Means not sharing the same superscript are different. ($P < 0.001$) within each column

[1] Each number is mean of three replicates, the protein, lactose and ash are expressed as percent of total solids ± standard deviation

[2] SM = skim milk powder (control)

[3] UF = ultrafiltration 4:1

[4] DF2, DF3 and DF4 = Second, third and fourth diafiltration, respectively.

[5] DF4-E = Fourth diafiltration with ethanol.

Table 3.3. Changes in the mineral concentration as a function of the milk processing by ultrafiltration and diafiltration.

	Minerals content (mg/g protein)[1]				
Treatment	Ca	P	K	Na	Mg
SM[2]	35.3 ± 0.20^{a}	29.0 ± 2.60^{a}	39.4 ± 2.54^{a}	12.9 ± 0.40^{a}	3.20 ± 0.07^{a}
UF[3]	$30.4 \pm 2.39^{ab*}$	$20.9 \pm 2.07^{b***}$	$15.3 \pm 2.62^{b***}$	$5.85 \pm 0.30^{b***}$	$1.86 \pm 0.19^{b***}$
DF2[4]	$29.4 \pm 0.68^{b*}$	$17.3 \pm 0.73^{bc***}$	$5.33 \pm 0.55^{c***}$	$2.70 \pm 0.07^{c***}$	$1.41 \pm 0.03^{bc***}$
DF3	27.4 ± 2.20^{b}	16.4 ± 0.66^{c}	3.72 ± 0.38^{c}	$2.03 \pm 0.08^{cd***}$	1.24 ± 0.12^{c}
DF4	27.9 ± 1.85^{b}	16.9 ± 0.86^{c}	3.01 ± 0.18^{c}	1.69 ± 0.16^{d}	1.20 ± 0.15^{c}
DF4-E[5]	26.9 ± 5.16^{b}	15.5 ± 0.75^{c}	2.76 ± 0.09^{c}	1.51 ± 0.05^{d}	1.17 ± 0.17^{c}

[a,b,c,d,e] Means not sharing the same superscript are different. [*](P<.05), [***](*P* <.001) within each column

[1] Each number is mean of three replicates ± standard deviation

[2] SM = skim milk powder (control)

[3] UF = ultrafiltration 4:1

[4] DF2, DF3 and DF4 = Second, third and fourth diafiltration, respectively.

[5] DF4-E = Fourth diafiltration with ethanol.

Table 3.4. **The content of undenatured whey proteins (UWP) in low and high heated skim milk and milk protein concentrate powders produced by ultrafiltration and diafiltration.**

	LT (72.6°C/16 sec)	HT (90°C/5 min)
	mg UWP (α-la+βlg)/g TMP[1]	
SM[2]	133.6[a]	4.14[b]
UF[3]	132.3[a]	6.10[b]
DF2[4]	130.8[a]	3.26[b]
DF3	143.6[a]	4.30[b]
DF4	139.7[a]	4.21[b]
DF4-E[5]	131.6[a]	2.76[b]

[a,b] Means not sharing the same superscript are different. ($P < 0.001$) within column

[1] Each number is mean of two replicates UWP = undenatured whey proteins, TMP = total milk protein

[2] SM = skim milk powder (control)

[3] UF = ultrafiltration 4:1

[4] DF2, DF3 and DF4 = Second, third and fourth diafiltration, respectively. [5] DF4 = Fourth diafiltration with ethanol.

The manufacturing procedure of the powders (i.e. composition) influences the moisture sorption isotherms. In this study, the low and high heated skim milk powder and milk protein concentrates powders manufactured by ultrafiltration and/or diafiltration, at A_w 0.9 held only between 22 to 27 g of H_2O per 100g of solids. These values are lower compared to 45 to 60g of H_2O per 100g of solids associated with spray dried milk powders (Spiess *et al.*, 1983).

In the data there is no clear breakpoint evident in the sorption isotherms of low and high heated milk protein concentrates. This could be attributed to the lactose being in a crystalline state (Labuza, 1984). This results in smooth isotherm, as observed in this study, rather than the sharp drop associated with an amorphous/glass state which is observed in high lactose powders (whey powders) between A_w 0.3 to 0.5, or in retentate powders at a A_w of 0.58 (Ozimek *et al*, 1992)

The difference between the values of UF retentate powders observed by Ozimek *et al.* (1992) and those presented in this study (Figures 3.2 and 3.3) for milk protein concentrate powders may be a result of lactose being removed by diafiltration. Modifications of the sorption isotherm curve of many whey and milk powders can be explained by the existence of a mixture of amorphous lactose and crystalline lactose. Kinsella *et al.* (1986) noted that cottage cheese containing 56% lactose showed a number of discontinuities from A_w 0.26 to 0.49 due to the various states of lactose crystallization equilibria.

Using the BET equation, the monolayer moisture content (Vm g/100g of solids), energy content (c) and surface moisture sorption area (SA m^2/g of solids) were calculated

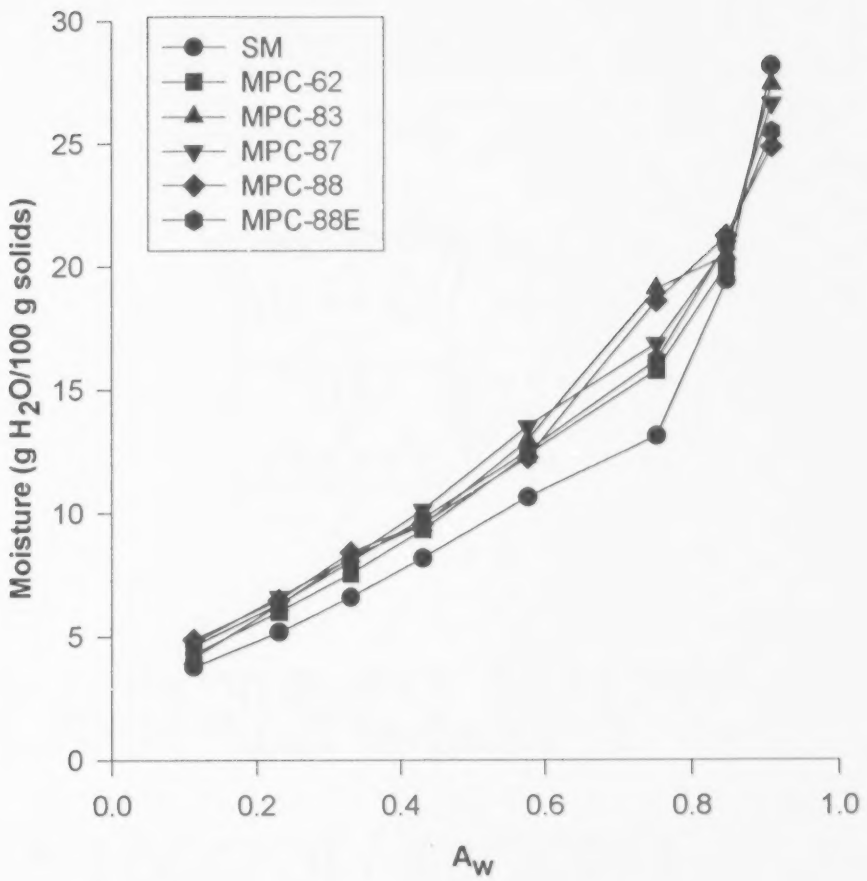

Figure 3.2. **Water sorption isotherms for freeze dried low heated milk protein concentrate powders produced by ultrafiltration and diafiltration and skim milk(control) at 20°C**

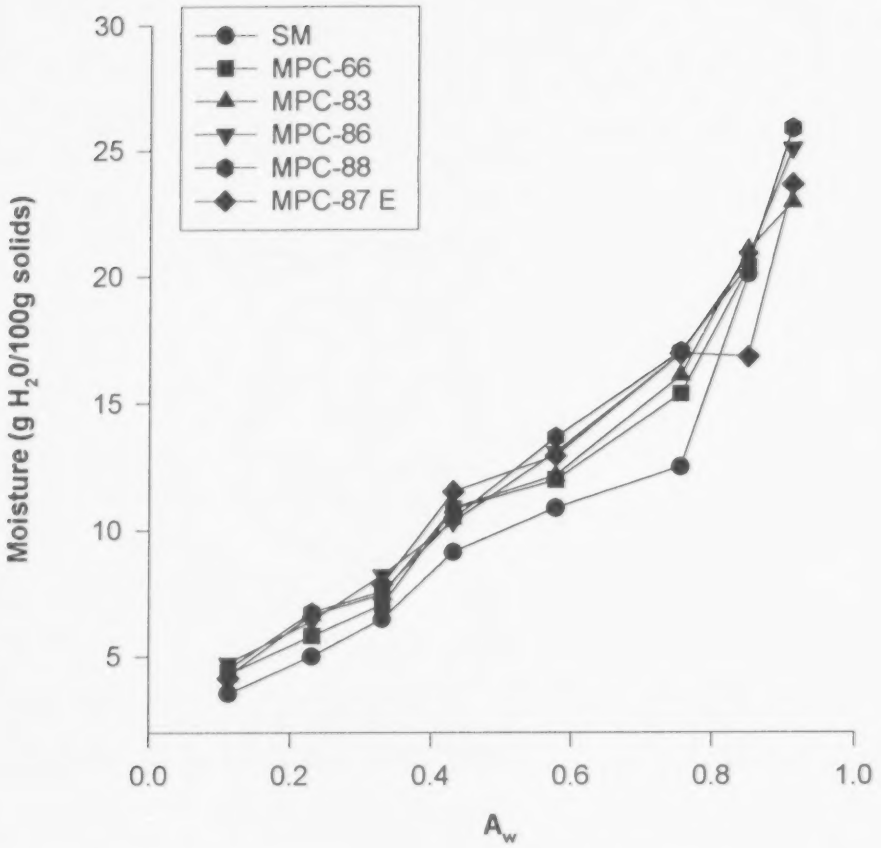

Figure 3.3. **Water sorption isotherms for freeze dried high heated milk protein concentrate powders produced by ultrafiltration and diafiltration and skim milk(control) at 20°C.**

for SM (control) and MPC powders produced by ultrafiltration and diafiltration. These are illustrated in Table 3.5. The monolayer moisture contents for low heat treated samples ranged between 5.7 and 6.2 g/100g of solids and were lower compared to the values for high heated samples which were between 6.5 and 7.3 g/100g of solids.

A similar pattern was observed for the surface area of SM and MPC powders produced by ultrafiltration. The values observed from the low and high heated MPC powders, illustrated in Table 3.5., range from 203.5 to 219.8 m^2/g of total solids and 232.8 and 261.2 m^2/g, respectively. This may be explained because the whey proteins in the high heated samples are denatured and have a greater number of polar binding sites on the available surface area. In the case of the lower heated samples, higher protein/protein and protein/sugar interactions causes losses of charge and polar groups, reduction in surface area, changes in surface topography and a marked reduction in porosity (Gregg *et al.*, 1981).

3.3.4. Surface Hydrophobicity

Fluorescence intensity of protein solution increased as ANS molecules bind to specific binding sites on protein molecules. Figures 3.4. and 3.5. shows a typical set of titration curves obtained from low and high heated skim milk and milk protein concentrates. The surface hydrophobicity (S_0) of low and high heated milk protein concentrates is illustrated in Table 3.6. Higher ($P<0.001$) surface hydrophobicity values were observed in the high heat milk protein concentrates. These higher values are due to protein denaturation which may lead to the unfolding of the molecule and thus exposing hydrophobic regions. The effect of processing on hydrophobicity was evaluated by

comparing samples from each step of the process. The hydrophobicity results were not affected by processing. The surface hydrophobicity was not changed ($P>0.001$) by ultrafiltration and diafiltration.

Table 3.5 The BET monolayer moisture concentration and surface area of milk protein concentrate powders produced by ultrafiltration and diafiltration.

	BET (Aw[10] =0.113-0.4) Low temperature			BET (Aw[10] =0.113-0.4) High temperature		
TMT[1]	Vm[6] (g/100g DM)	SA[7] (m^2/g DM[9])	c[8]	Vm[6] (g/100g DM[9])	SA[7] (m^2/g DM[9])	c[8]
MPC-62[3]	5.74	203.5	14.8	6.56	232.8	8.35
MPC-83[4]	6.08	215.7	12.9	6.20	237.7	9.31
MPC-87	6.20	219.8	15.9	6.32	225.8	13.5
MPC-88	5.85	207.5	22.8	6.33	224.3	12.7
MPC-88[5]	6.00	212.9	16.1	7.37	261.2	7.11

[1]TMT = Identity of each sample [2]

[3] MPC-62= milk protein concentrate after ultrafiltration 4:1, [4] MPC-83, 87, 88, = milk protein concentrate after second, third and fourth diafiltration, respectively.

[5] MPC-88 = milk protein concentrate, fourth diafiltration with ethanol.

[6] Vm = monolayer capacity, [7] SA = surface sorption area, [8] c = thermodynamic parameters of BET

[9] DM = dry matter [10]Aw = water activity

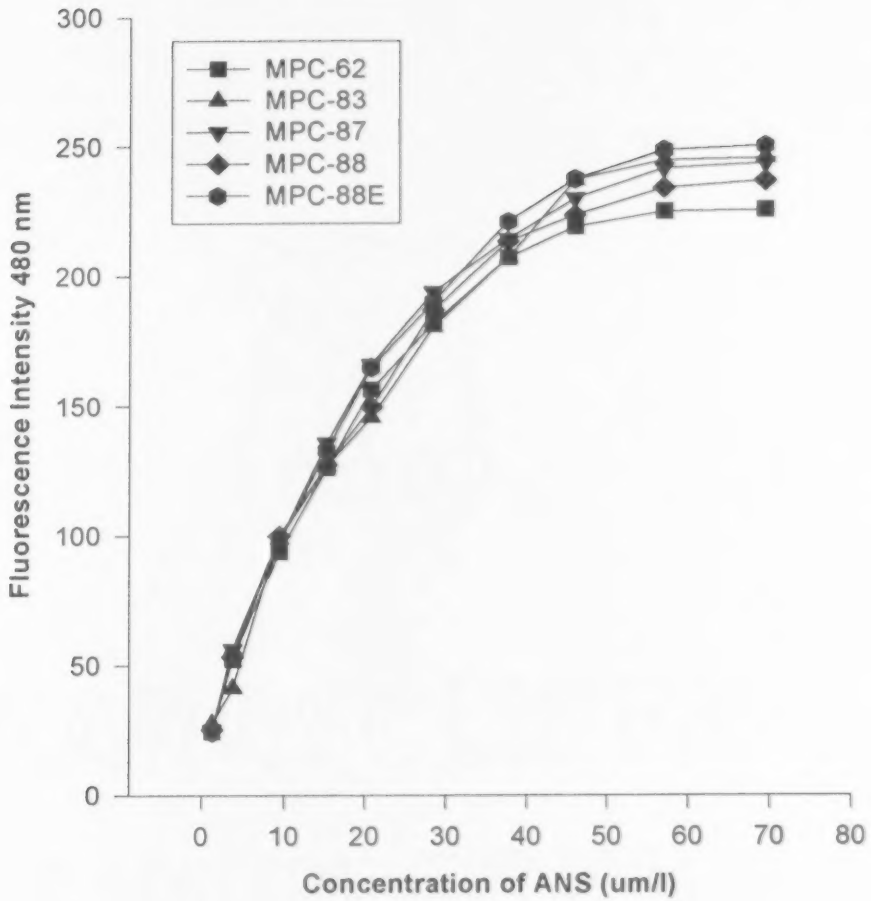

Figure 3.4. **Spectrofluorometric titration curves for low heated milk protein concentrate powders produced by ultrafiltration and diafiltration and skim milk powder (control).**

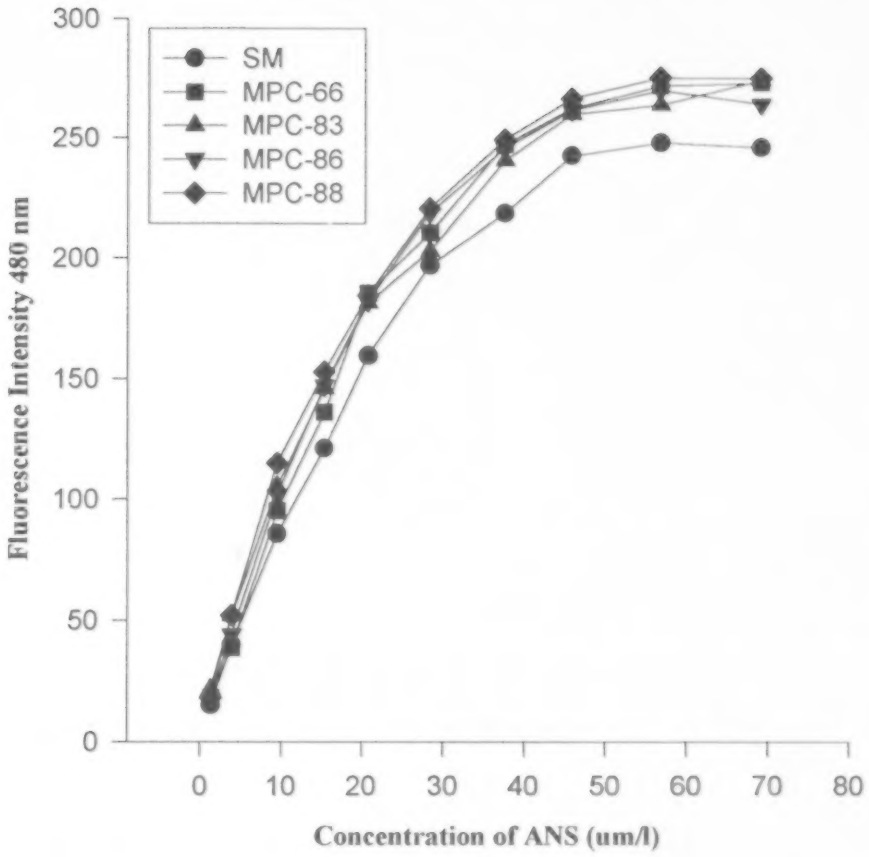

Figure 3.5. **Spectrofluorometric titration curves for high heated milk protein concentrate powders produced by ultrafiltration and diafiltration and skim milk powder (control).**

Table 3.6. **Surface Hydrophobicity (S_o) for the low and high heated skim milk powder (control) and milk protein concentrate powders produced by ultrafiltration and diafiltration.**

	LT (72.6°C/16 sec)	HT (90°C/5 min)
	S_o	
SM[2]	1061.9[a]	1313.4[b]
MPC-62[3]	1134.7[a]	1477.8[b]
MPC-83[4]	1250.1[a]	1412.7[b]
MPC-87	1122.3[a]	1307.8[b]
MPC-88	1150.5[a]	1422.7[b]
MPC-88[5]	1168.7[a]	1287.2[b]

[a,b] Means not sharing the same superscript are significantly different. ($P < 0.001$)

[1] Each number is mean of two replicates

[2] SM = skim milk powder (control)

[3] MPC-62= milk protein concentrate after ultrafiltration 4:1, [4] MPC-83, 87, 88, = milk protein concentrate after second, third and fourth diafiltration, respectively.[5] MPC-88 = milk protein concentrate after fourth diafiltration with ethanol.

3.4. Conclusions

Low and high heated milk protein concentrates of different composition (MPC-62 to MPC-88 and MPC-66 to MPC-88, respectively)[1] were manufactured by ultrafiltration and diafiltration. Lactose was removed from 58 to 2% (low temperature) and 59 to 1% (high temperature) during these processes. The distribution of minerals in each milk protein concentrate was analyzed. The degree of whey protein denaturation was then determined in order to determine their effect on water sorption isotherms and surface hydrophobicity. Finally, surface hydrophobicity and water sorption isotherms were studied.

The results highlighted that ultrafiltration and/or diafiltration can be used to manufacture milk protein concentrate powders with different protein, mineral and lactose contents. These different products can then be targeted for a wide range of applications.

It was established that different heat treatments of skim milk prior to ultrafiltration and/or diafiltration cause whey protein denaturation. As a result, it can be concluded that heat treatment causes structural changes in protein which will affect water sorption isotherms and surface hydrophobicity.

The information generated by this study adds to the body of knowledge necessary for defining shelf-life, packaging, and other processing parameters. For example, the calculated monolayer and critical moisture content can be used to establish the packaging and storage parameters for the long term storage of UF milk retentate powders. However,

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[1] The number after MPC or MP indicates the actual protein content.

further studies should be conducted to determine the correlation between surface hydrophobicity and other functional properties as they relate to new product development

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Chapter 4

4. Heat Stability of UF Retentate Powders

4.1. Introduction

The production of milk protein concentrates (MPC) by ultrafiltration (UF) technique has gained acceptance in the industry settings as well as in research applications. More recent applications for UF include cheesemaking, whey concentrates and retentate powders (Maubois and Mocquot, 1974). UF is used for preconcentration of milk prior to its use in continuous cheesemaking and yogurt operations, recovery and purification of whey proteins from dairy effluent streams, concentration of milk prior to transport, and standardization of milk for total solids and protein contents (Kosikowski, 1986).

Ultrafiltration involves moving the fluid (i.e. milk or whey) across semi-permeable membranes under pressure. The pressure, coupled with the distinct pour sizes in the membranes, facilitates the removal of water while retaining the majority of solids in the retentate. Compounds of lower molecular weight, such as soluble minerals, are lost to the permeate as well as is most of the lactose.

MPC are designed to be used in many food applications that use heat treatment. Therefore, a particularly important functional property of MPC is its thermal stability. UF MPC results in the increased incorporation of heat labile proteins into an environment of high casein concentration. Heating the MPC also serves to improve microbiological safety (UF would also serve to concentrate biological contamination), to inactivate enzymes for long term storage, or impart heat-induced functionality (eg. increased viscosity, gelation,

or improved water binding). The understanding of the heat stability of the heat labile whey proteins and caseins in these concentrated system is essential.

Since UF is, by definition, a fractionation unit operation, discriminating between retained and rejected constituents solely on the basis of molecular weight allows the passage of low molecular weight constituents (lactose, mineral salts, non-protein nitrogen) to the waste stream (Cheryan, 1986). The residual presence of some of these microsolutes (in particular mineral salts), the concentration ratio of the UF operation, the concentrate pH, and the type and temperature history of the dairy fluid to be concentrated, are all important factors expected to influence the thermal stability of the final concentrated system (Singh and Creamer, 1992).

MPC represent a complex system of which the heat stability is not well defined. Recent work focused on the kinetics of protein denaturation, although cited work has been limited mostly to simple one component systems (Hillier *et al.*, 1979 and Dannenberg *et al.*, 1986).

In the manufacturing of dairy products such as cheese, milk powder and milk protein concentrates as well as in other foods, heat treatment is often involved. There is very little information available in the literature on the effect of the behavior of retentates when heated. Minerals such as Ca and Mg, play an important role in heat stability of milk during processing of milk products (Singh and Creamer, 1992). For example, the presence of soluble calcium is a reason for faster enzymatic coagulation of milk during cheese making.

It is thought that there is a correlation between the change in the heat stability of milk and loss of minerals. Morrissey (1969) reported significant effects of soluble calcium

phosphate on heat coagulation time (HCT) suggesting that the deposition of calcium phosphate on a casein/β-lactoglobulin complex destabilizes the micelle complexes leading to protein precipitation. It can also be expected that ultrafiltration/diafiltration of milk cause significant changes in the composition and functionality of MPC.

The objective of this study was to determine the stability of milk protein concentrate powders produced by ultrafiltered skim milk. The effect of sterilization temperatures (120, 130 and 140 °C) and pH in the range from 6.4 to 7.4 on heat stability of milk protein concentrate powders were also studied.

4.2. Materials and Methods

4.2.1. Preparation of UF Retentate Powders

A laboratory DDS-20 unit with twenty 0.018 m^2 membranes with molecular weight cut-off of 25,00 Daltons was used for separation and concentration. Commercial HTST pasteurized skim milk was ultrafiltered at 50 ± 1°C, with inlet and outlet pressures of 400 and 320 kPa, respectively. The extent of and ultrafiltration process was measured by the volumetric concentration factor (VCF) where:

VCF = Volume of original feed / Volume of final concentrate

Part of the skim milk was kept and used as a control which was expressed as VCF 1:1.

4.2.2. Analytical Methods

4.2.2.1. Composition of Powders

The samples of skim milk powder (control) and retentate were analyzed for dry matter, protein, fat, lactose, ash, Ca, P, Mg, Na and K. Dry matter was determined by the

hot air oven method (AOAC, 1990). Total protein and casein were determined by the macro-Kjeldahl method according to AOAC methods (1990). Fat was determined by the Mojonnier procedure (Atherton and Newlander, 1977). Lactose was analyzed using the sulfuric and phenol colorimetric method (Lawrence, 1968). Ash was determined by igniting in a muffle furnace at 550 °C. For mineral content, P was determined by the phosphomolybdate colorimetric method after ashing using the International Dairy Federation (IDF) standard method (IDF, 1990), Ca and Mg by Atomic Absorption Spectrophotometry, and Na by Fame Emission Spectrophotometry using a Perkin-Elmer 400 Spectrophotometer.

4.2.3. Heat Stability Test

The pH of bulk concentrated milk samples (about 40 ml) was adjusted to values between 6.4 and 7.4 at 0.1 intervals using 2N HCL or 2N NaOH. The stock solutions were held at 4°C for at least one hour before testing to allow for adequate equilibration. Heat coagulation times were determined at three different temperatures (120, 130 and 140°C) using a thermostatically controlled hot oil bath according to the standard subjective heat stability test described by Davies and White (1966). Coagulation was observed with an aided eye.

4.3. Results And Discussions

4.3.1. Composition of UF Retentate Powders

Chemical composition of skim milk retentate of different volumetric concentration factors are given in Table 4.1.. The content of total protein, casein, lactose, and minerals in skim milk was in a rage similar to that observed in normal skim milk. During

concentration of skim milk by ultrafiltration, the content of casein increased in proportion to the concentration factor (VCF). In practice, the measurement of protein content in retentate could be accurately used to determine the concentration factor of the UF retentate. However, the percentage of lactose removed from skim milk decreased to 19.9 % during ultrafiltration to volume concentration factor 5:1 (Table 4.1.).

There was a linear increase in mineral content (Ca, P, Mg, K and Na) as the concentration factor increased, which is reflected by the increase in ash. However, the increase in mineral content was less extensive than that of milk proteins. Thus, the content of individual minerals, expressed in mg/g of casein, decreased continuously to VCF 3 (Table 4.2). Further concentration of milk up to VCF 5 did not affect the proportion of Ca, P, Mg and casein. This indicates that soluble forms of Ca, P and Mg were removed from milk during a three-fold concentration. In the retentate, the remaining colloidal form of minerals was casein-calcium (magnesium)-phosphate complex. Further reduction of the mineral content in the colloidal form could be carried out by decreasing the pH of milk and diafiltration process (Quist *et al.*, 1987).

Table 4.1. Chemical composition of milk protein concentrate powders manufactured by ultrafiltration.

VCF[1]	Moisture (%)	Total Protein (% DM[2])	Casein (% DM[2])	Lactose (% DM[2])	Ash (% DM[2])	Fat (% DM[2])
2:1	4.00	57.2	39.1	39.0	7.98	2.23
3:1	3.25	59.9	48.2	31.4	8.00	3.51
4:1	4.38	64.8	50.8	25.1	7.99	4.32
5:1	3.47	68.7	52.9	19.9	7.90	5.26

[1]VCF - volume concentration factor
[2]DM - dry matter

Table 4.2 The content of minerals of milk protein concentrate powders manufactured by ultrafiltration.

	Minerals content (mg/g casein)[1]				
(VCF)	Ca	P	Mg	Na	K
1	53.0 ± 2.79[a]	44.9 ± 4,88[a]	4.0± 0.14[a]	14.8 ± 1.87[a]	62.0 ± 1.17[a]
2	45.5 ± 0.06[b]	34.9 ± 9.48[b]	3.0± 0.07[b]	8.5 ± 0.79[b]	33.0 ± 1.03[b]
3	41.3 ± 1.61[b]	29.3 ± 0.27[c]	2.3 ± 0.16[c]	5.7 ± 1.01[c]	23.1 ± 1.55[c]
4	42.9 ±1.52[b]	28.7 ± 0.40[c]	2.2 ± 0.08[c]	4.7± 0.98[c]	19.0 ± 1.69[c,d]
5	43.0 ± 1.75[b]	27.3 ± 0.84[c]	2.1± 0.12[c]	3.9± 0.57[c]	15.6± 0.56[d]

[1]mean ± SD

[a,b,c,d] Different letters within each column indicate differences $P<0.05$

4.3.2. Heat Stability of Milk Protein Concentrate Powders

Table 4.3 and Figure 4.1 show the heat coagulation times of skim milk and milk protein concentrate powders at different temperatures and pH values.

The curves illustrated in Figure 4.1. are almost identical to those reported by Rose (1961a), showing an HCT maximum at about pH 6.8 and a minimum at about pH 6.9-7.0. Milk that produces these kinds of curves were classified as Type A by Tessier and Rose (1964) in order to distinguish them from milk that shows an approximately linear increase in HCT with a rise in pH and which were classified as Type B milks. Approximately 80% of milk from individual cows in Canada show Type A characteristics (Tessier and Rose, 1964).

In addition to exhibiting pronounced maxima and minima HCT, Figure 4.1 illustrates that heat coagulation times fell sharply with increased temperature. Also, all the curves maintain a similar general shape without overlapping. However, as temperature increases, the minima become less sharply defined. While the minima are clearly defined at pH 6.9-7.1., Tables 4.4 and 4.5 and Figures 4.2 and 4.3. A similar maxima is shown (at about pH 6.7-6.8) for 2:1 and 3:1 concentrations. However, the maxima for a 4:1 concentration (Table 4.6 and Figure 4.4) are broad and essentially eliminated at higher temperatures, and beginning at a concentration of 2:1 (Figure 4.2), and clearly established at 3:1 (Figure 4.3) and 4:1 (Figure 4.4), the nimima are all essentially lost. In Figure 4.2 (2:1 concentration) at 120 and 130°C the minima are spread out over a pH range of 6.9-7.2 and the minima is lost completely at 140°C. In Figures 4.3 and 4.4 (3:1 and 4:1 concentrations) no minima are apparent except for the point at 130 °C and pH 7.1 in

Figure 4.4. All curves clearly maintain similar general shapes without significant overlap for different temperatures at a given concentration. There is quite obviously no increase in HCT.

Figures 4.5, 4,6 and 4.7 each show the HCT characteristics of the control and all three concentrations at a single constant temperature. As above, in all cases, as temperature rises, HCT decreases and as concentration rises, HCT also decreases.

Some samples were tested fresh from ultrafiltration while other were not tested until a day or two after. This was done in an effort to determine the effect of storage on the results of heat stability determinations. Retentate samples of 3:1 and 4:1 were stored at 4°C for a week and were then compared to samples which were ultrafiltered and tested on the same day (Figures 4.8 and 4.9). While there are some graphically visual differences, the HCT scale (y-axis) is small. Using a Paired Difference Student's t-Test, it was found that there was no significant difference in the heat coagulation time for fresh or onw week old samples ($P>0.1$).

4.4. Conclusions

In conclusion, the data presented show that the concentration of skim milk by ultrafiltration has a direct effect on heat stability in that the heat coagulation time decreases with increasing concentration. Milk concentrates produced by ultrafiltration showed Type A behaviour. The effect of pH on heat stability of concentrated milks by ultrafiltration is very similar to that of unconcentrated milk although milk retentates remain unstable at pH above 6.8. Practical knowledge of heat stability of concentrates by ultrafiltration will allow the industry to manipulate processing parameters when applied to other food systems. However further studies of the kinetics of heat induced changes are needed in order to better understand the mechanisms of heat coagulation in UF milk protein concentrates.

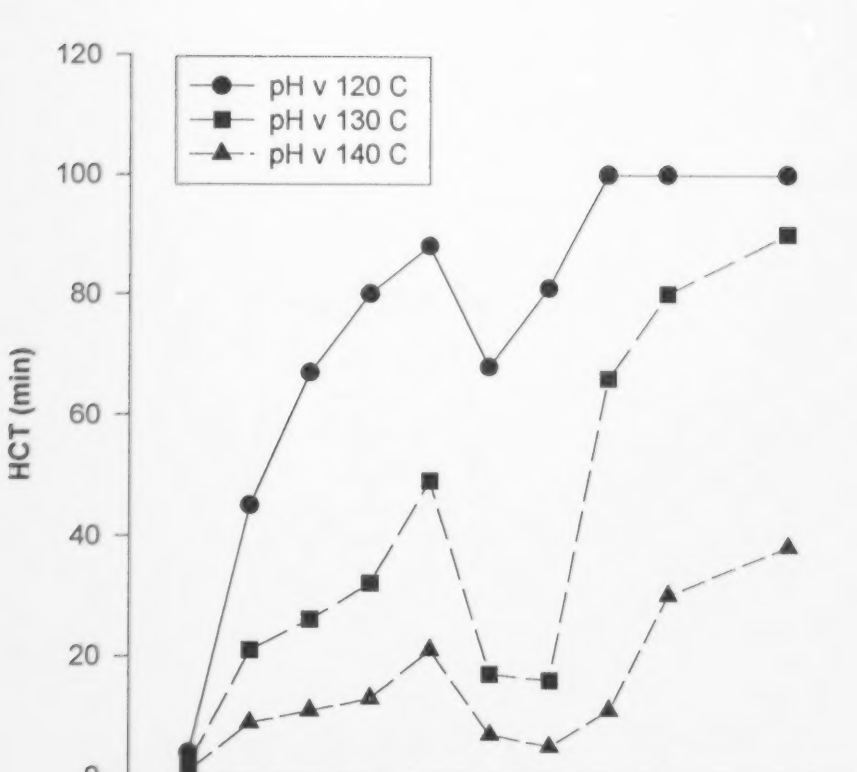

Figure 4.1. **Heat coagulation time (minutes) vs pH at 120, 130 and 140°C for 1:1 skim milk.**

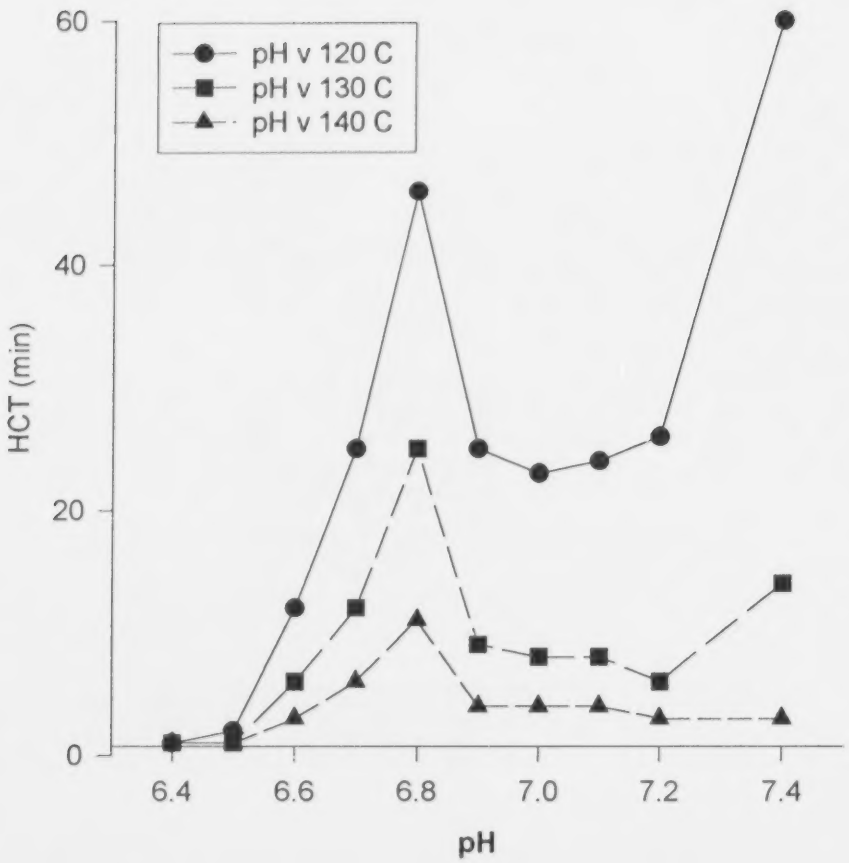

Figure 4.2. **Heat coagulation time (minutes) vs pH at 120, 130 and 140°C for 2:1 skim milk.**

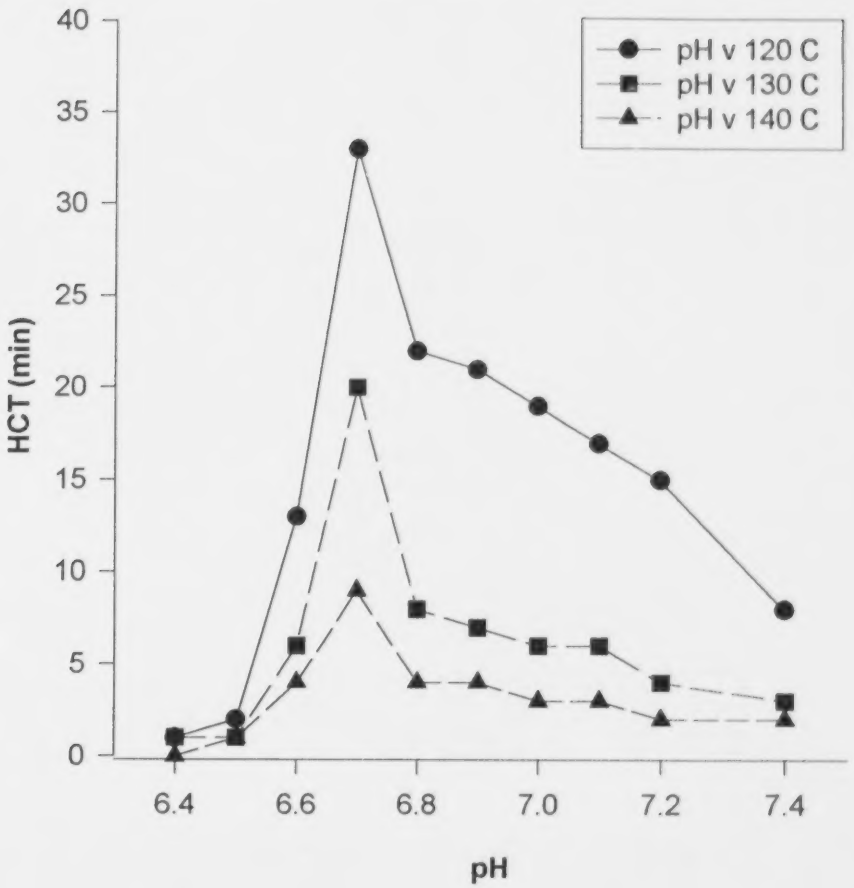

Figure 4.3. **Heat coagulation time (minutes) vs pH at 120, 130 and 140°C for 3:1 skim milk.**

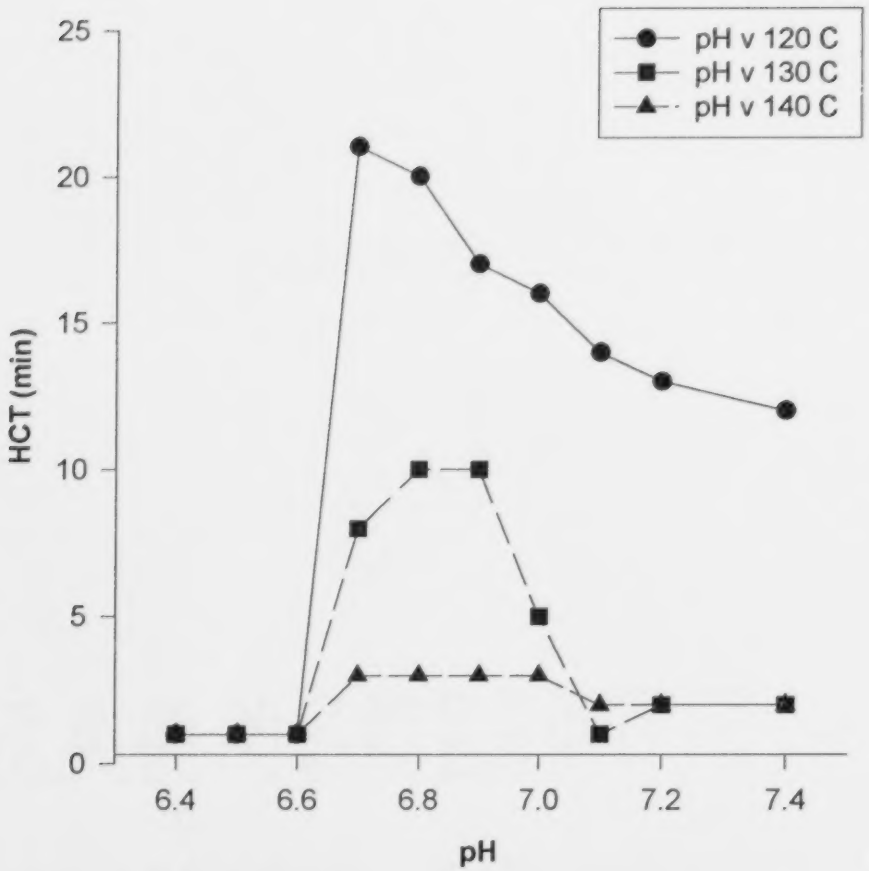

Figure 4.4. **Heat coagulation time (minutes) vs pH at 120, 130 and 140°C for 4:1 skim milk.**

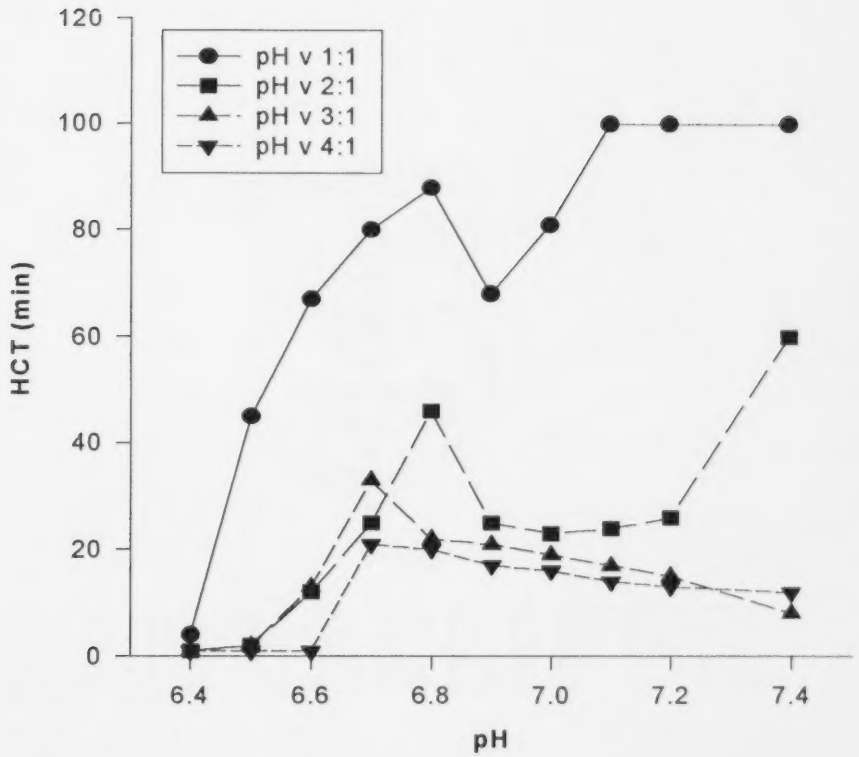

Figure 4.5. **Heat coagulation time (minutes) vs pH at 120°C for 1:1, 2:1, 3:1, and 4:1 skim milk.**

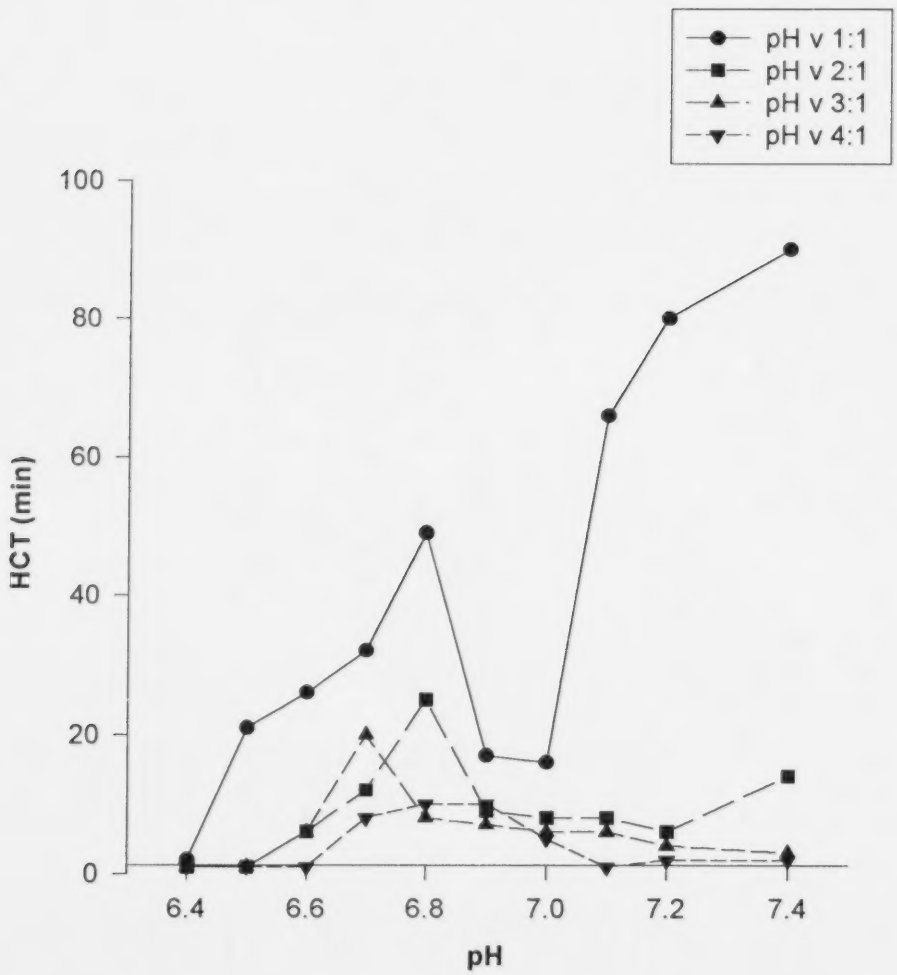

Figure 4.6. **Heat coagulation time (minutes) vs pH at 130°C for 1:1, 2:1, 3:1, and 4:1 skim milk.**

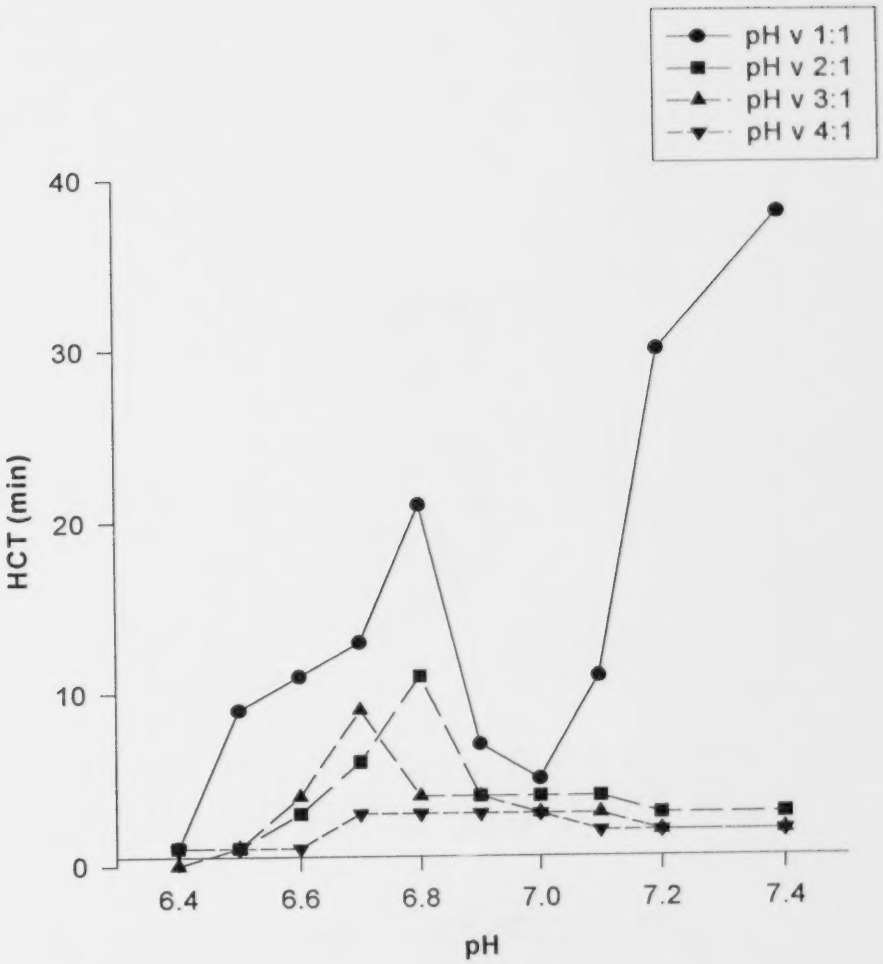

Figure 4.7. **Heat coagulation time (minutes) vs pH at 140°C for 1:1, 2:1, 3:1, and 4:1 skim milk.**

Table 4.3. Heat coagulation time at 120, 130 and 140°C for 1:1 skim milk at pH values between 6.4 and 7.4.

pH	120°C	130°C	140°C
	Heat coagulation time (minutes)		
6.4	4	2	1
6.5	45	21	9
6.6	67	26	11
6.7	80	32	13
6.8	88	49	21
6.9	68	17	7
7.0	81	16	5
7.1	>100	66	11
7.2	>100	80	30
7.4	>100	>90	38

Table 4.4. Heat coagulation time at 120, 130 and 140°C for 2:1 skim milk at pH values between 6.4 and 7.4.

pH	120°C	130°C	140°C
	Heat coagulation time (minutes)		
6.4	1	1	1
6.5	2	1	1
6.6	12	6	3
6.7	25	12	6
6.8	46	25	11
6.9	25	9	4
7.0	23	8	4
7.1	24	8	4
7.2	26	6	3
7.4	>60	14	3

Table 4.5. Heat coagulation time at 120, 130 and 140°C for 3:1 skim milk at pH values between 6.4 and 7.4.

pH	120°C	130°C	140°C
	Heat coagulation time (minutes)		
6.4	1	1	0
6.5	2	1	1
6.6	13	6	4
6.7	33	20	9
6.8	22	8	4
6.9	21	7	4
7.0	19	6	3
7.1	17	6	3
7.2	15	4	2
7.4	8	3	2

Table 4.6. Heat coagulation time at 120, 130 and 140°C for 4:1 skim milk at pH values between 6.4 and 7.4.

pH	120°C	130°C	140°C
	Heat coagulation time (minutes)		
6.4	1	1	1
6.5	1	1	1
6.6	1	1	1
6.7	21	8	3
6.8	20	10	3
6.9	17	10	3
7.0	16	5	3
7.1	14	1	2
7.2	13	2	2
7.4	12	2	2

Table 4.7. Heat coagulation time for fresh and 1 week old samples of 3:1 and 4:1 retentates.

pH	HCT (A) Old (x_1)	HCT (B) Fresh (x_2)	d	d^2
6.4	1	1	0	0
6.5	1	5	-4	16
6.6	6	1	5	25
6.7	20	8	12	144
6.8	8	9	-1	1
6.9	7	6	1	1
7.0	6	5	1	1
7.1	6	5	1	1
7.2	4	4	0	0
7.4	3	3	0	0

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Chapter 5

Concluding Remarks

5. Summary of Research Findings

The purpose of this study was to manufacture milk protein concentrate powders with different levels of protein and lactose by ultrafiltration and diafiltration and then to 1) study their chemical composition and properties; 2) determine whey protein denaturation and to establish the heat classification of milk protein concentrates; 3) evaluate water sorption isotherms and surface hydrophobicity of milk protein concentrate powders; and 4) determine the heat stability of various retentates manufactured by ultrafiltration.

Through ultrafiltration and diafiltration it was possible to manufacture low and high heated skim milk protein concentrates with MPC-62, MPC-83 and MPC-88 resulting in powders having lower concentrations of lactose. This allows for the tailoring of milk protein concentrates for specific industrial applications. In addition, those milk protein concentrate powders with the lowest levels of lactose can be used in populations where lactose malabsorption is prevalent. Four batch diafiltration it was necessary to produce the milk protein concentrates with lactose level below ca. 2% and protein above 87%.

Heat classification of milk protein concentrates was determined and proposed by measuring the amount of undenatured whey protein (UWP) present in milk protein concentrate powders and the results are expressed in mg of undenatured whey protein per g of total protein. Low and high heated milk protein concentrates contained on average 136 and 4.40 mg UWP per g of total protein respectively. This information is of value in

that it can be used by the industry to determine the most appropriate application of these powders.

Individual mineral concentration on a dry matter (mg/g of total protein) changed during ultrafiltration and diafiltration. Calcium decreased ($P<0.05$) during ultrafiltration although during diafiltration there was not decrease in concentration. Four fold ultrafiltration and second diafiltration caused decrease ($P<0.001$) in the concentration of Na and K. Phosphorus and magnesium decreased ($P<0.001$) during ultrafiltration and second diafiltration. Further diafiltration did not affect the proportion of Ca, P and Mg. This is a function of the proportion of minerals bound to protein-calcium(magnesium)-phosphate complex.

Water sorption isotherms of milk protein concentrates produced a smooth sigmoid Type II isotherms, according to the BET classification. In general, equilibrium moisture content (EMC) was similar for all products although the EMC for skim milk powder was lower than that of milk protein concentrate powders with higher protein concentration.

At higher water activities ($A_w>0.65$) there was a steep increase in moisture concentration for skim powder and milk protein concentrates due to moisture adsorption by salts and/or gradual dissolution of crystalline lactose. There was no difference between low and high heated skim milk powder and milk protein concentrates in the EMC.

The manufacturing procedure of the powders (i.e. composition) influences the moisture sorption isotherms. Low and high heated skim milk powder and milk protein concentrates powders manufactured by ultrafiltration and/or diafiltration, at A_w 0.9 held only between 22 to 27 g compared to the 45 to 60g of H_2O per 100g of solids associated with spray dried milk powders

Significantly ($P<0.001$) higher surface hydrophobicity values were observed in the high heat milk protein concentrates. The surface hydrophobicity results were not affected by ultrafiltration and diafiltration. Moreover, the surface hydrophobicity was affected by heat treatment.

Different heat treatments of skim milk prior to ultrafiltration and/or diafiltration cause whey protein denaturation. As a result, heat treatment causes structural changes in protein which will affect water sorption isotherms and surface hydrophobicity.

In the study focusing on heat stability the data demonstrate that the concentration of skim milk by ultrafiltration has a direct effect on heat stability in that the heat coagulation time decreases with increasing concentration. Milk concentrates produced by ultrafiltration showed a Type A behaviour. The effect of pH on heat stability of concentrated milks by ultrafiltration is very similar to that of unconcentrated milk although milk retentates remain unstable at pH above 6.8.

6. Recommendations for Future Study

The information from this study adds to the body of knowledge associated with the development of value added dairy products and for processing parameters such as defining shelf-life and packaging. For example, the calculated monolayer and critical moisture content can be used to establish the packaging and storage parameters for the long term storage of UF milk retentate powders. Practical knowledge of heat stability of concentrates by ultrafiltration will allowe the industry to manipulate processing parameters when applied to other food systems.

However, further study is necessary to determine the full extent to which ultrafiltration and diafiltration can be of benefit to the food industry. Further research into the kinetics of heat-induced changes are needed in order to better understand the mechanisms of heat coagulation in milk protein concentrate powders. Correlations between surface hydrophobicity and other functional properties as they relate to product development need to be determined. Milk protein concentrates powders need to be further studied in order to ascertain additional specifications in relation to the products to be manufactured.

The functional properties of milk protein concentrates also need to be studied in relation to the particular food systems (i.e. bakery, meat, etc.) to which they are to be applied. It is also critical that the feasibility of large scale ultrafiltration and diafiltration processes be undertaken in order to determine cost effectiveness.